Nutrient Polishing and Carbon Sequestration

Yingying Tang

Nutrient Polishing and Carbon Sequestration by Macrophyte-Dominated Wetlands

Yingying Tang

Nutrient polishing and carbon sequestration by macrophyte-dominated wetlands

Yingying Tang

ISBN: 978-94-6233-476-2

Cover design: LaTeXStudio

Photos: Emma, Ernandes Sobreira Oliveira Junior and Yingying Tang

Lay-out by: LaTeXStudio

Printed by: Gildeprint, Enschede

The research presented in this thesis was performed at Department of Aquatic Ecology & Environmental Biology, Institute for Water and Wetland Research, Faculty of Science, Radboud University Nijmegen, the Netherlands. Yingying Tang was supported by a grant from China Scholarship Council.

Nutrient polishing and carbon sequestration by macrophyte-dominated wetlands

Proefschrift

ter verkrijging van de graad van doctor

aan de Radboud Universiteit Nijmegen

op gezag van de rector magnificus prof. dr. J.H.J.M. van Krieken,

volgens besluit van het college van decanen

in het openbaar te verdedigen

op dinsdag 6 december 2016

om 14.30 uur precies

door

Yingying Tang

geboren te Anhui, China,

op 5 July 1984

Promotoren:

Prof. L.P.M. Lamers

Prof. A.J.P. Smolders

Prof. J.G.M. Roelofs

Copromotor:

Dr. M.M.L. Van Kempen

Manuscriptcommissie:

Prof. J.C.J.M. De Kroon

Prof. C. Mariani

Prof. X. Li (East China Normal University, Shanghai)

Nutrient polishing and carbon sequestration by macrophyte-dominated wetlands

Doctoral Thesis

to obtain the degree of doctor

from Radboud University Nijmegen

on the authority of the Rector Magnificus prof. dr. J.H.J.M. van Krieken,

according to the decision of the Council of Deans

to be defended in public on Tuesday, December 6, 2016

at 2:30 p.m. precisely

by

Yingying Tang

born in Anhui, China,

on July 5, 1984

Supervisors:

Prof. L.P.M. Lamers

Prof. A.J.P. Smolders

Prof. J.G.M. Roelofs

Co-supervisor:

Dr. M.M.L. Van Kempen

Manuscript Committee:

Prof. J.C.J.M. De Kroon

Prof. C. Mariani

Prof. X. Li (East China Normal University, Shanghai)

Contents

Chapter 1

General introduction

Yingying Tang

Natural and constructed wetlands

Wetlands, such as marshes, fens, bogs and lakes, exist along the interface between terrestrial and aquatic environments, their nature, biodiversity and functioning depending on climate, geohydrological setting and management. They can be either natural or artificial, permanent or temporary, with water that is static or flowing (Navid, 1989; Finlayson & Moser, 1991; Mitsch & Gosselink, 2007). Wetlands are among the biologically most productive ecosystems in the world (Costanza et al., 1997; Moreno-Mateos et al., 2012) and provide multiple ecosystem services including biodiversity, protection of shorelines, flood mitigation, improvement of water quality and water supply, and carbon (C) sequestration (Mitsch & Gosselink, 2000; Millennium Ecosystem Assessment, 2005; Clarkson et al., 2013).

It is, however, estimated that worldwide about 50% of all wetlands ecosystems have been lost due to human activities including drainage of land for agricultural purposes and construction of dams for hydropower generation or for the creation of water reservoirs (OECD, 1996; Zedler & Kercher, 2005; Mitsch & Gosselink, 2007). For a large number of the remaining wetlands, eutrophication is a major environmental issue (Smith, 2003; Anderson et al., 2014), leading to deterioration of water quality, harmful algal and cyanobacterial blooms, and a serious reduction in biodiversity (Pretty et al., 2003; Verhoeven et al., 2006; Conley et al., 2009; Le et al., 2010; Lamers et al., 2015). Eutrophication of wetlands can be caused by direct discharge of domestic, agricultural and industrial wastewater (external eutrophication; Roelofs (1991)), or by increased nutrient (including phosphorus, P) mobilization from the soils into water bodies (internal eutrophication), especially for eutrophic soils (Howarth et al., 2000; Smolders et al., 2006). To compensate for the loss of natural wetlands, drained wetlands are being restored and new wetlands are being created. In addition, wetlands are specifically constructed for the removal of pollutants from wastewater (Mitsch et al., 2005; Wolf et al., 2011). Essentially, all these different wetland ecosystems show similar biogeochemical and ecological mechanisms that are differentially regulated by variations in soil and water quality, and in biological communities.

Wetland soils and eutrophication

Soils, major and active components of wetlands, can serve as sinks, sources and transformers of chemical contaminants such as nutrients and heavy metals, thereby having a significant influence on water quality and wetland productivity (Reddy & DeLaune, 2008). This is particularly the case for shallow wetlands (Lamers et al., 2015). Based on their organic matter contents, soils can be divided into organic and mineral soils, which is important given the pivotal role of microorganisms using organic compounds in biogeochemical cycling. Besides this, several physicochemical features including the temperature, level of aeration, nutrient availability, pH, soil texture and organic matter decomposability play key roles. For the research presented in this thesis, peat,

Figure 1.1. The five different aquatic macrophytes used as model species in this thesis. From left to right: *Ceratophyllum demersum*, *Chara hispida*, and *Myriophyllum spicatum* (all submerged), *Azolla filiculoides*, *Eichhornia crassipes* (floating). Source: `ian.umces.edu/symbols/`.

clay, organic sludge (highly decomposed underwater sediment) and sandy soils were chosen to represent the variation in wetland soils. Clay soils show a stronger binding affinity for water and nutrients due to their small particle size generating a large surface area. Peat soils (relatively undecomposed organic soils), and highly decomposed underwater soils (sludge) also show a higher cation exchange capacity than sandy soils, in general. Depending on their eutrophication legacy, all different soil types can show large variations in nutrient availability. In combination with other physicochemical features related to nutrient mobilization such as redox potential, this results in large differences in vegetation type and ecosystem productivity.

Wetland vegetation

Wetland plants, an integral part of both natural and constructed wetlands, play an important role in the biogeochemical cycling of C and nutrients, and resulting ecosystem services. The recreation or rehabilitation of an indigenous vegetation community is therefore required to restore degraded or destroyed wetlands (Brown & Bedford, 1997). For wetlands that are constructed as treatment plants, vegetation can be used to assimilate and accumulate nutrients, which can subsequently be removed from the wetlands by regular harvesting (Li et al., 2009; Tang et al., 2016a). Macrophytes (plants and macroalgae) growing in wetlands can be divided into three categories based on their life form: emergent aquatic macrophytes (not included in this thesis), floating-leaved aquatic macrophytes, and submerged aquatic macrophytes (Cronk & Fennessy, 2001; Brix, 2003). In this thesis, *Ceratophyllum demersum*, *Chara hispida*, and *Myriophyllum spicatum* were chosen a model species to represent submerged macrophytes, while *Azolla filiculoides* and *Eichhornia crassipes* were studied as model floating macrophytes (Figure 1.1).

Ceratophyllum demersum

C. demersum (commonly known as Hornwort or Coontail) is a non-rooted, submerged aquatic macrophyte, which can still extract nutrients from the soils by forming modified leaves (rhizoids) to anchor to the soils (Toetz, 1974; Keskinkan et al., 2004). However, it mainly takes up nutrients

directly from water bodies, and can therefore improve water quality (Denny, 1972; Lombardo & Cooke, 2003). Its finely dissected or feathery leaves potentially enhance its capacity for assimilation of nutrients and C (Ornes & Sajwan, 1993; Lombardo & Cooke, 2003). In addition, *C. demersum* shows a high vegetative propagation and biomass production, even under a moderate nutrient supply (Aravind et al., 2009; Chorom et al., 2012).

Chara hispida

C. hispida, a submerged aquatic macro-alga in the Charophyta division (known as stoneworts), possesses fine rhizoids that can anchor to the soils and can consequently absorb nutrients from the soils (Box et al., 1985; Box, 1986). In addition, *Chara* spp. can also take up nutrients from surface water through both branchlets and stems. Although high P concentrations can indirectly suppress the growth of *C. hispida* due to shading by phytoplankton (Forsberg, 1964; Blindow, 1992), its relatively slow decomposition rates, and its ability to overwinter and prolong nutrient storage in plant biomass, make *C. hispida* act as an efficient nutrient sink under oligo- and mesotrophic conditions (Kufel & Kufel, 2002; Rodrigo et al., 2007).

Myriophyllum spicatum

M. spicatum (Eurasian watermilfoil), a submerged aquatic macrophyte, can tolerate a wide range of environmental conditions including pH, temperature, and salinity. Although submerged macrophytes are generally less productive than terrestrial and emegent macrophytes due to the limited diffusion rate and availability of carbon dioxide (CO_2) in water (Grace & Wetzel, 1978; Nichols & Shaw, 1986), *M. spicatum* can use bicarbonate (HCO_3^-) in addition to CO_2 as a C source for photosynthesis (Adams et al., 1978). It can grow fast and spread quickly by seed production, by rhizome production, and paticularly by fragmentation (Nichols & Keeney, 1976; Smith & Barko, 1990). In additon, it takes up P from the sediment by its roots and by its leaves (Carignan & Kalff, 1980; Smith & Barko, 1990), and can flourish in mesotrophic and (slightly) eutrophic water (Aiken et al., 1979; Melchior, 1997), which make *M. spicatum* an ideal candidate for use in constructed wetlands for both wastewater and nutrient-rich sediment polishing.

Azolla filiculoides

A. filiculoides (Water fern), a floating aquatic macrophyte, can quickly colonize P-rich waters to form dense mats. Therefore, this species can effectively be used as a biosorbent for wastewater treatment alleviating eutrophication of aquatic ecosystems. In addition, due to its very high growth rates, *A. filiculoides* has enormous potential to sequestrate atomospheric CO_2, thereby reducing net greenhouse gas (GHG) emissions from wetlands. Its symbiosis with the dinitrogen(N_2)-fixing cyanobacteria *Anabaena azollae* enables it to grow well under nitrogen-deficient

conditions, provided that the availability of phosphorus is sufficiently high (Hill, 1998; Sanyahumbi et al., 1998; Van Kempen et al., 2013). After its harvest, *A. filiculoides* can be used as a green manure to fertilize crops such as rice paddies, and as food for animals (Moore, 1969; Peters & Meeks, 1989). The species was introduced to Europe in 1880 (West, 1953), after its extinction on this continent during the last glacial periods (Ekman, 1999).

Eichhornia crassipes

E. crassipes (Water hyacinth), a floating aquatic macrophyte, has been widely used for wastewater treatment in natural and constructed wetlands due to its wide tolerance to pollution, high morphological plasticity, high nutrient absorption capacity, ease of propagation (mainly via clonal reproduction), and rapid growth (Muramoto & Oki, 1983; Méthy et al., 1990; Ding et al., 1994; Ebel et al., 2007). Furthermore, because of its high growth rates, *E. crassipes* can sequester large amounts of CO_2. Apart from absorbing nutrients and sequestrating C, *E. crassipes* can also serve as a green fertilizer, and as fodder for animals due to its high protein content and richness of many kinds of vitamins (Isarankura-Na-Ayudhya et al., 2007; Chang & Cheng, 2016). Very recently (in July 2016), *E. crassipes* was included in the EU list of invasive alien species to prevent the introduction and spread of this species (EU, 2016).

Biogeochemical processes in wetlands related to nutrient and C cycling

There is a suite of biogeochemical mechanisms involved in the processes of C sequestration and nutrient removal in natural and constructed wetlands, including photosysthesis, decomposition, methane (CH_4) production and oxidation (C cycling), nitrogen (N) in- and output through groundwater and surface water, atmospheric deposition, N_2 fixation, plant N uptake, nitrification, denitrification, volatilization, DNRA (dissimilatory nitrate reduction to ammonium), anammox (anaerobic ammonium oxidation) (N cycling), P in and output, plant P uptake, soil precipitation and adsorption, and iron oxidation and reduction (P cycling) (Howard-Williams, 1985; Bowden, 1987; Moore et al., 1998).

C cycling

Wetlands play a substantial role in the global C cycling, due to their large soil C storage, major source of atmospheric CH_4, potential net sink of atmospheric CO_2, sediment deposition, and plant biomass (Gorham, 1991; Blodau, 2002; Bridgham et al., 2006; Lamers et al., 2015). Many wetlands are net CO_2 sinks due to the prevailing waterlogged and anaerobic conditions that slow down decomposition rates and accrete organic matter in soils (Bridges, 1978; Ström et al.,

2005). However, anaerobic conditions at the same time stimulate the production of CH_4, a greenhouse gas with a 34 times greater global warming potential (GWP) than CO_2 on a mass basis over a 100 year period (Moore et al., 1998; Stocker et al., 2013). There are multiple gas transport pathways that regulate CH_4 emissions into the atmosphere in macrophyte-dominated wetlands. CH_4 that is mainly produced in anoxic soils can be exported by any of the following three pathways: diffusion, ebullition (bubbles from soils), or transport via the aerenchyma of vascular plants (Bartlett et al., 1988; Tokida et al., 2007; Bastviken et al., 2008). Once diffusive CH_4 fluxes reaches oxic-anoxic boundaries, a large part of the CH_4 can be oxidized to CO_2 by methane-oxidizing bacteria, thereby reducing GHG emissions in terms of GWP (Heilman & Carlton, 2001; Bastviken et al., 2004; Kosten et al., 2016). However, CH_4 transported by bubbles or through roots and aerenchymatous tissues of macrophytes can escape CH_4 oxidation and subsequently directly be emitted into the atmosphere (Tokida et al., 2007; Natchimuthu et al., 2014). Given that wetlands both emit CH_4 and CO_2 (and other GHG such as nitrous oxide), and sequester CO_2 from the atmosphere, a combination of these two offsetting processes will determine whether a wetland ecosystem acts as a GHG sink or a GHG source.

N cycling

N cycling in wetlands embodies the in- and output of N by surface water, groundwater and atmosphere, and a series of abiotic and biotic transformations, including N uptake by plants and algae, mineralization, N fixation, ammonia volatilization, denitrification, and anammox. N input from surface water and groundwater has strongly increased in many wetlands, leading to eutrophication, deterioration of drinking water quality, changes in biotic communities, and the accumulation of toxins such as ammonia and ammonium (Smolders et al., 2010; Zaman & Blennerhassett, 2010; Lamers et al., 2015). Biological N_2 fixtion represents an important natural N input to wetland ecosystems and can maintain ecosystem productivity when fixed N supplies are depleted (Howarth et al., 1988; Reddy & DeLaune, 2008). Atmospheric N deposition has changed from a relatively unimportant N resource to a sometimes dominant N source, due to the increase in anthropogenic N emissions induced by human demands for food and energy (Galloway et al., 2008; Bobbink et al., 2010). Denitrification is the principal process by which N can ultimately be removed from wetlands and also the main pathway by which nitrous oxide (N_2O), another greenhouse gas having a GWP of 298 times CO_2 over a 100 year time scale, is emitted to the atmosphere (Weier et al., 1993; Stocker et al., 2013). In addtion to coupled nitrification/denitrification, nitrogen losses from wetlands can also be attributed to the annamox process, anaerobic ammonium oxidation coupled to nitrite reduction (Erler et al., 2008; Zhu et al., 2010). The importance of ammonia volatilization depends on the pH of the water column, and this process may be important for submerged macrophyte-dominated wetlands due to high pH during the day ($pH > 8.5$) induced by photosynthesis of submerged macrophytes (Reddy et al., 1984; Vymazal, 2007).

P cycling

As a result of anthropogenic changes, P input into wetlands by surface water, groundwater and (to a small degree) atmosphere has increased, resulting in shifts in vegetation composition and resulting faunal communities (Grime, 1974; Lamers et al., 2015). Next to changing biotic communities by changing competitive interactions, a large part of this P can be bound to wetland sediments. Abiotic P immobilization and mobilization by wetland soils is regulated by a suite of soil characteristics including pH, the amount and type of clay minerals, redox potential, iron (Fe), calcium (Ca) and aluminum (Al) content of soils, and organic matter content (Khalid et al., 1977; Smolders et al., 2006; Reddy & DeLaune, 2008; Lamers et al., 2015). High pH can cause P liberation from Fe-bound and Al-bound P primarily due to ligand exchange reactions in which hydroxide ions (OH^-) replace orthophosphate (PO_4^{3-}), while P can be immobilized by co-precipitation by calcium carbonate ($CaCO_3$) or Ca^{2+} ions at high pH (Boström et al., 1988; Reddy & DeLaune, 2008). In soils, P can also be fixed to iron (III) oxides and hydroxides. Due to its role as an alternative electron donor in microbial Fe(III) reduction (Küsel et al., 2002; Chacon et al., 2006), Fe cycling is tightly linked to both P and C cycling. The input of labile organic matter can lead to mobilization of P by the reduction of ferric iron (Fe (III)) to ferrous iron (Fe (II)). Fe (II) has a lower P-binding efficiency than Fe (III) (Patrick Jr. & Khalid, 1974; Smolders et al., 2001; Emsens et al., 2016), leading to P release from soils to the water layer. Increased rates of microbial sulphate reduction can also lead to P mobilization, as sulphide (produced by sulphate reduction) interferes with the iron-phosphorus cycle, by reducing iron(III) (hydr)oxides and iron(III) phosphates. Subsequently, highly insoluble iron sulphides (FeS_x) are formed, reducing the availability of Fe to bind P (Smolders et al., 2006). Soils with high non-labile organic matter contents (peat soils) or high clay content can strongly contribute to P retention due to their high concentration of P-sorption sites (Ramulu et al., 1967; Harter, 1969).

Main objectives and outline of this thesis

The main goal of this thesis was to study and optimize nutrient uptake and C sequestration in macrophyte-dominated wetlands. To achieve this, a series of laboratory and mesocosm experiments was carried out to study the roles of both soil and vegetation type, and focusing on both biological and biogeochemical processes related to these wetland services.

It is well known that P can easily become mobilized from soils to the overlying water after the restoration of wetlands and the construction of permanent or riparian (temporarily flooded) wetlands on former agricultural lands. As this leads to eutrophication problems, it is urgent to find an easy tool to predict P release rates after short-term flooding that is widely applicable to a range of different soil types. In **Chapter 2**, we combined experimental work on P mobilization rates with the measurement of a suite of soil characteristics for a large set of different soils ranging from sandy

to highly organic. In addition, we determined the temperature dependence of P-mobilization, to be able to correct for temperature related seasonal variations. The results can be used as a standard method to support land and nature managers in their decision-making concerning the optimal locations for the construction and restoration of wetlands.

Next to acting as nutrient sources, soils can also serve as nutrient sinks in both natural and constructed wetlands. In **Chapter 3**, we used a full factorial outdoor mesocosm design to test the effects of different nutrient loadings on the resulting distribution of nutrients over different compartments: water layer, plant biomass and soil. In the experiment we compared different plant species (*C. demersum*, *C. hispida*, *M. spicatum* and *A. filiculoides*) in combination with different soil types (peat, peaty clay and clay soils). The outcome of the results enables the selection of efficient macrophytes species for wastewater polishing depending on nutrient loading rates, which is essential for decision support in water management using constructed wetlands for nutrient removal by biomass harvesting.

In addition to nutrient removal, macrophytes also play an important role in the regulation of GHG fluxes. In **Chapter 4**, we determined the effects of plant density and sediment rooting on nutrient dynamics and GHG fluxes of *E. crassipes* vegetation, using a full factorial, controlled indoor aquarium experiment. Our findings highlight the importance of incorporating plant growing conditions when assessing regional greenhouse gas fluxes for wetlands.

Chapter 5 studies the role of sediment composition in P mobilization and GHG emission in wetlands dominated by *A. filiculoides*. The mobilization of Fe and P into the water layer, their assimilation by *Azolla*, and GHG emissions were determined for two inundated agricultural soils with different Fe reduction rates, in a full factorial, indoor aquarium experiment. We showed that organic matter composition, rather than Fe or P content, could explain Fe reduction rates, which in turn strongly regulate the emissions of GHG, dissolved organic carbon (DOC) and P from *Azolla* dominated wetlands.

Finally, in **Chapter 6**, a conceptual design is proposed to optimize nutrient removal and C sequestration in wetlands, based on the synthesis of the results of the laboratory and mesocosm experiments described in the previous chapters. In addition, the implications for water management, land management, and resource management are elaborated in this chapter.

Chapter 2

A tool for easily predicting short-term phosphorus mobilization from flooded soils

Yingying Tang, Monique M.L. Van Kempen, Tjisse Van der Heide, Johan J.A. Manschot,

Jan G.M. Roelofs, Leon P.M. Lamers, Alfons J.P. Smolders

Ecological Engineering 94 (2016): 1-6

Abstract

The construction and restoration of riparian (temporarily flooded) wetlands as water storage and flood protection areas plays a central role in climate-adaptive water management. In general, arable and ex-arable lands are used for this type of water storage. However, inundation may lead to problems, as excess phosphorus (P) stored in these soils may be released and result in the eutrophication of the overlying surface waters. Clearly, water and nature managers need to be able to determine for which areas temporary water storage would be a feasible option without causing eutrophication problems. Here, using a controlled experimental approach, a simple predictive tool for the P mobilization rates from soils upon short-term inundation has been developed. A large suite of soil characteristics and P mobilization rates were determined during flooding for different soil types (peat and sand), at two different depths to mimic topsoil removal (topsoils and soils from −30 to −60 cm below ground level), and at two temperatures to test seasonal influence (8°C and 18°C). Increasing the temperature from 8 to 18°C almost tripled P mobilization rates, but the variation could not be linked to any of the soil characteristics measured - average Q_{10} (temperature coefficient) values were 2.8 (2.9 for peaty soils, 2.6 for sandy soils). Although P mobilization was related to P saturation of amorphic Fe, water-extractable P was found to be by far the best predictor for short-term P mobilization rates, explaining 86.9% of the variation. The predictive tool for P mobilization after short-term rewetting is simple, low-cost and widely applicable, and can support water managers during their decision-making processes concerning the optimal location for the construction of water storage areas, the restoration of riparian wetlands, and the combinational use of different ecosystem services.

Introduction

To meet the challenges of population growth and climate change, landscapes are increasingly being designed for multi-functional use, combining ecosystem services to enhance land use efficiency. It is predicted that the frequency of severe flooding events in Europe will increase in the future due to the intensification of the global hydrological cycle resulting from climate change (IPCC, 2014; Alfieri et al., 2015). To deal with this, temporary water storage areas are essential during peak discharge periods to prevent flooding of urban areas. In addition, new riparian wetland areas are being developed to improve water retention and water purification, to reduce greenhouse gas emissions, to increase nature restoration, and for recreational use. Such new areas for water storage, flood protection and/or riparian wetland restoration are frequently planned for both arable and formerly arable lands. As the extensive use of fertilizer and manure during farming generally have exceeded the output in primary production, large amounts of phosphorus (P) have accumulated in these soils (Richardson, 1985; Barberis et al., 1995; Pant & Reddy, 2003; Smolders et al., 2008; Geurts et al., 2011). Flooding of these P rich lands often causes eutrophication of the overlying water due to flood-induced mobilization of P resulting from oxygen (O_2) depletion in the soil (Richardson, 1985; Lamers et al., 1998; Lamers et al., 2001; Loeb et al., 2007). The nutrient release and the accumulation of reduced chemicals, such as ammonium and sulfide, leads to algal and cyanobacterial blooms, a die-off of target vegetation, a decrease in biodiversity, and an overall loss of wetland environmental quality (Pretty et al., 2003; Smolders et al., 2006; Conley et al., 2009; Lamers et al., 2015). Clearly, these effects undermine the intended multi-functional land use.

For water security and environmental quality reasons, water managers need to be able to select temporary water storage areas wisely. However, currently there exists no simple investigative tool to aid in the selection of suitable locations, and an easy and cost-effective method to classify the potential P mobilization rates of different soils during short-term flooding events would be of great value so that lands with low P mobilization rates can be selected as areas for temporary water storage and retention and/or restoration of riparian wetlands.

It is well known that changes in iron (Fe) reduction rates (Mortimer, 1941; Ponnamperuma, 1984; Richardson, 1985), sulfate (SO_4^{2-}) reduction rates (Caraco et al., 1989; Moore & Reddy, 1994; Lamers et al., 1998), decomposition rates (McLatchey & Reddy, 1998), and interactions among Fe, sulfur (S), P and O_2 (Smolders et al., 2006; Loeb et al., 2007; Cusell et al., 2013) strongly determine the actual P mobilization rates of soils upon inundation. In the present study, a suite of soil characteristics related to these mechanisms were determined for a large number of agricultural soils, in relation to their P mobilization rates to the overlying water. The aim was to find a reliable, simple indicator that could accurately predict P mobilization during temporary flooding for a wide range of soils.

As P mobilization may depend on organic matter content (McLatchey & Reddy, 1998), different soils were used in this study to create a large range of soil organic matter contents. Since topsoil removal is an important measure when creating temporary storage basins, specifically to reduce P availability during flooding (Van Dijk et al., 2004; Emsens et al., 2015), deeper soil layers (−30 to −60 cm) were included to test P mobilization after topsoil removal. In addition, to test the influence of seasonal variation in temperature on P mobilization (Boers & Van Hese, 1988; Liikanen et al., 2002) two temperatures were used (8°C and 18°C).

In order to find a reliable, simple indicator that could accurately predict P mobilization during temporary flooding for a wide range of soils, this study needed to answer the following questions: (i) which soil characteristics can be used for the prediction of P mobilization rates during short-term flooding; (ii) does the predictability of P release differ for soils with different organic matter contents, as well as for topsoil versus deeper soil; and (iii) how does temperature affect P mobilization rates.

Materials and methods

Fieldwork

Soil samples were collected using standard sharpened stainless steel cylinders (coring method) in October 2013 in the following areas (see Supplementary Table 2.1 for all coordinates): Zuidplas (strongly decomposed peat; 51°59′N, 4°39′E; 5 locations), Burkmeer (strongly decomposed peat; 52°25′N, 4°59′E; 3 locations), Ilperveld (strongly decomposed peat; 52°26′N, 4°55′E; 4 locations), Wormer-Jisperveld (strongly decomposed peat; 52°31′N, 4°49′E; 4 locations), Stelkampsveld (sand; 52°06′N, 6°28′E; 2 locations) and Hallerlaak (sand; 52°04′N, 6°22′E; 5 locations) (n = 23). At each of the 23 locations soils were sampled at 0 to −30 cm and −30 to −60 cm depth, with the deeper soil samples mimicking the situation after top-soil removal. The soil samples were put in plastic bags and kept at 4°C until further analyses.

Soil analyses

Fresh soil samples were volume-weighed and subsequently dried (48 h, 60°C) after which they were re-weighed to determine bulk density, and subsequently grinded with a mortar and pestle. Organic matter content was determined by loss on ignition (4h, 550°C. 200 mg of dry soil was digested in a microwave oven (MLS-1200 Mega, Milestone Inc., Sorisole, Italy) using 4 mL 65% HNO_3 and 1mL 30% H_2O_2 to determine total sediment Fe and P concentrations. Digested solutions were then analyzed with inductively coupled plasma-optical emission spectrometry (ICP-OES; IRIS Intrepid II, Thermo Electron Corporation, Franklin, MA, USA). Water extracts were derived by incubating 17.5 g fresh soil in 50 mL Milli-Q for 2 hours at 105 RPM,

and Olsen P extracts (plant available phosphorus) were derived by incubating 3 g of dry soil in 60 mL $NaHCO_3$ for 0.5 hour at 105 RPM (Olsen et al., 1954). Oxalate extracts were used to determine the concentrations of amorphous Fe and Fe-bound and aluminum- (Al-) bound P by incubating fresh soil material, corresponding to approximately 2.5 g dry weight in a 50 mL mixture of ammonium oxalate monohydrate and oxalic acid dehydrate in the dark for 2 hours at 105 RPM (Houba et al., 1989). Extracts were collected in vacuum flasks using rhizons, after which the samples were decanted in 10 mL test tubes. The Olsen and oxalate extracts were diluted 5 and 4 times, respectively, before they were decanted in the test tubes. 0.1 mL of 65% HNO_3 was added to the test tubes (10 ml) containing the water extractions to avoid precipitation of redox sensitive compounds. Samples were kept in the dark at 4°C until further chemical analyses.

Experimental set-up

At the start of the experiment 400 mL of each soil sample was placed in 1L infusion bottles. Next, 600 mL of a standardized solution containing 1000 μmol L^{-1} $NaHCO_3$, 1000 μmol L^{-1} $CaCl_2.2H_2O$, 250 μmol L^{-1} KCl and 500 μmol L^{-1} $MgSO_4.7H_2O$ were carefully added to all bottles. The open bottles were placed in the dark, either at 8°C ('low temperature', L), or at 18°C ('high temperature', H). The experiment comprised 122 infusion bottles in total (18°C: 42 peat and 28 sand soils; 8°C: 29 peat and 23 sand soils), and the incubation was for 6 weeks. During the experiment 20 mL surface water samples were collected twice weekly for the first three weeks and once every week for the last three weeks. After each sampling, 20 mL fresh surface water solution was added to compensate for the loss of the sampling volume for which was corrected in the calculation of P mobilization rates.

Analyses

Concentrations of PO_4 in the surface water were measured colorimetrically (Bran & Luebbe Autoanalyzer III, Norderstedt, Germany) using ammonium molybdate (Geurts et al., 2008). Total P, Fe, S were analyzed by ICP-OES (see above). At the end of the experiment, O_2 concentrations in the surface water were measured in all bottles with an optical oxygen probe (Hach LDO, Hach, Loveland, CO, U.S.A.).

Calculation of P release from sediments and Q_{10}

For the time span which showed a linear increase of surface water PO_4 concentration over time, P mobilization rates from soils into the overlying water were calculated by linear regression. The effect of increasing the temperature by 10°C on biogeochemical rates, also known as the Q_{10} temperature coefficient, was determined by dividing mobilization rates at 18°C by those at 8°C.

Statistical analyses

In order to meet the assumption that residuals fit a normal distribution, P mobilization rates (response variable) and most of all selected soil characteristics (predictor variables) were transformed by $\log_{10}$ (variable) or by $\log_{10}$ (variable + 1) (Bartlett, 1947) in cases where the lowest value of a variable was below one. For P mobilization rates, models with all possible subsets of all selected soil characteristics were fitted. Coefficients of each predictor variable across all considered models were averaged and their 95% confidence intervals (CIs) were calculated (Burnham & Anderson, 2002). An effect of predictor variable on the response variable was considered significant if CIs did not include zero. The relative importance (w) of each predictor variable was calculated by summing the weights of the Akaike information criterion (AICc: correction for small sample size) across all considered models, where a given predictor variable occurred (Burnham & Anderson, 2002), with 1 being the best predictor and 0 the worst. The influence of water-extractable P and temperature on P mobilization rates (both having a $w > 0.9$) was analyzed using linear regression models. All analyses were performed using the software program R (version 3.2.1; R development Core Team, 2015); model-averaged coefficients were tested by 'MuMIn' package (Bartoń, 2015). Q_{10} values were considered to correlate significantly with soil characteristics when $P < 0.05$.

Results

As bulk densities of soils differ depending on their origin, the units μmol L^{-1} or g L^{-1} soil fresh weight were used instead of μmol g^{-1} or g g^{-1} soil dry weight for selected soil characteristics. P mobilization rates (See Supplementary Table 2.2 for P mobilization rates and soil characteristics) were calculated by linear regression of surface water PO_4^{3-} concentrations at different times during short-term flooding, and expressed per unit soil surface.

Best predictors

P mobilization rates were best predicted by the combination of water-extractable P and temperature under aerobic surface water conditions (oxygen contents: 8.38 ± 0.09 mg L^{-1} and 9.52 ± 0.10 mg L^{-1} for 18°C and 8°C respectively), with $w > 0.9$ for both variables (Table 2.1). Moreover, 88.1% of the variation in P mobilization rates could be explained by constructing a linear model using only these two variables, and a model including only water-extractable P was already able to explain 86.9% (Table 2.2). After water-extractable P and temperature, organic matter content and Fe/P_{total} ratios had the greatest effect on P mobilization rates, with w being 0.59 and 0.50, respectively. Finally, soil depth and soil type showed much lower w values than the four predictor variables (Table 2.1).

Table 2.1. Model-averaged global regression coefficients and 95% of CIs from the model of P mobilization rates against soil characteristics showing Akaike weights (w) > 0.24; Coefficients are in bold where CIs do not overlap 0; Values of w represent the relative importance of the predictor variable based on summing weights of models where the given variable occurred; OM represents organic matter content; P_{water} represents water-extractable P.

Terms	Coefficient	Standard error	Lower CI	Upper CI	w
P_{water}	**0.732**	0.106	**0.521**	**0.943**	1
Temp H	0.170	0.370	−0.566	0.907	0.93
Temp L	0.011	0.370	−0.725	0.746	
OM	−0.118	0.142	−0.400	0.165	0.59
Fe/P_{total}	−0.094	0.145	−0.382	0.194	0.50
peat	0.251	0.497	−0.739	1.241	0.41
sand	0.173	0.416	−0.655	1.001	
S_{total}	0.051	0.102	−0.152	0.255	0.39
Fe/S_{total}	0.044	0.182	−0.318	0.406	0.36
P_{total}	0.030	0.127	−0.223	0.284	0.33
P_{olsen}	0.040	0.117	−0.194	0.273	0.33
Fe/P_{water}	−0.026	0.063	−0.151	0.099	0.32
$Fe/P_{oxalate}$	−0.002	0.108	−0.217	0.213	0.30
Top layer	0.132	0.393	−0.649	0.913	0.25
Deep layer	0.139	0.388	−0.633	0.912	

Table 2.2. Results from linear regression models of P mobilization rates in relation to water-extractable P and temperature, and water-extractable P only.

Explanatory terms in model	R^2	P	AICc
P_{water} + Temperature	0.881	0.000***	−14.517
P_{water}	0.869	0.000***	−11.021

Effects of best predictors

P mobilization rates correlated linearly ($P < 0.001$) with water-extractable P concentrations at both temperatures (Fig. 2.1) (Table 2.3). At 18°C 89.3% of the variation in P mobilization rates could be explained for all soils; at 8°C 86.5% of the variation in P mobilization rates could be explained for all soils (Table 2.3).

Temperature effect

The Q_{10} of soil P mobilization rates did not correlate ($P > 0.05$) with any of the selected soil characteristics, including organic matter contents (Fig. 2.2). Results show that most Q_{10} values

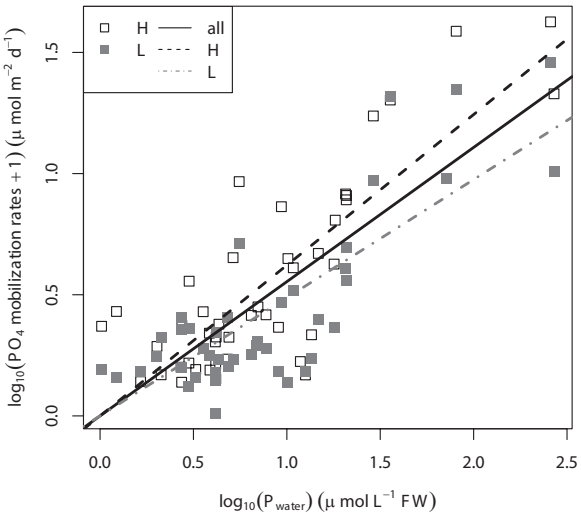


Figure 2.1. Correlations between P mobilization rates and water-extractable P concentrations for all temperatures, and for high (18°C; indicated H) and low temperatures (8°C; indicated L) separately.

Table 2.3. Results from the linear regression analyses of water-extractable P concentrations on P mobilization rates; y represents P mobilization rates; x represents water-extractable P concentrations; All represents all incubations at both temperatures; H (high temperature) represents incubations at 18°C; L (low temperature) represents incubations at 8°C.

models	formula	R^2	P
All	$\log_{10}(y+1) = 0.554 * \log_{10}(x)$	0.869	0.000***
H	$\log_{10}(y+1) = 0.622 * \log_{10}(x)$	0.893	0.000***
L	$\log_{10}(y+1) = 0.488 * \log_{10}(x)$	0.865	0.000***

remained in the range of 1–3 and that Q_{10} of soil P mobilization rates were on average 2.8, 2.9 and 2.6 for all, peat and sand soils, respectively.

Discussion

Prediction of flooding-induced P mobilization

P mobilization rates in the present study varied between 0.010 and 1.280 mg P m^{-2} d^{-1}. Of all selected soil characteristics, water-extractable P was the best predictor of P mobilization rates upon short-term flooding; 86.9% of the variation in P mobilization rates could be explained by this variable. Increasing the temperature from 8 to 18°C almost tripled P mobilization rates, but the variation could not be linked to any of the soil characteristics measured. In contrast to water-extractable P, total P concentration in soils could not be used to accurately predict P

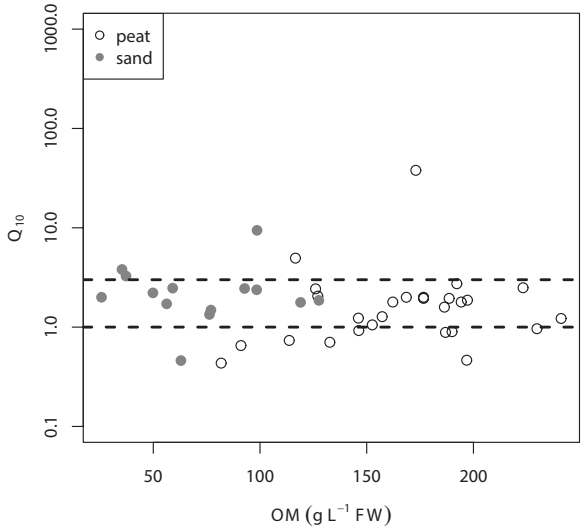


Figure 2.2. Correlations between Q_{10} and organic matter contents. Most Q_{10} values stayed in the range of 1–3 indicated by the dashed lines. Note the logarithmic scale of the y-axis.

mobilization rates. Based on P sorption and desorption characteristics, Hooda et al. (2000) similarly showed for clay and loam soils that next to Olsen P and P on Fe-coated paper, water-extractable P could predict P desorption. For a cluster of four areas differing in fertilization history, all with Spodosoil soil type. Pant and Reddy (2003) similarly showed that water-soluble P together with acid-extractable magnesium explained a large part of the variation in P desorption rates. Based on a large data set, the results show that water-extractable P can be used as a general, widely applicable and low-cost predictor that can be used by water managers and other natural resource managers.

For underwater soils, Fe plays an important role in modulating P mobilization to the overlying water layer (Jensen et al., 1992; Smolders et al., 2006; Smolders et al., 2010; Geurts et al., 2011), as oxidized Fe binds P at the soil-water interface (Einsele, 1938; Mortimer, 1941). For flooded soils, the same mechanism can be expected to play a role (Loeb et al., 2008). Under aerobic conditions, P is firmly bound by iron (III) oxides and hydroxides. After flooding, however, oxygen soon becomes depleted in the soil and Fe (III) is used as an alternative electron acceptor for the microbial oxidation of organic matter and reduced to the more mobile Fe (II) (Patrick Jr. & Khalid, 1974; Roy & Bickerton, 2014). P formerly bound to Fe (III) oxides and hydroxides becomes mobile and dissolves in the pore water (Loeb et al., 2007). Depending on whether the soil-water interface is aerobic or anaerobic, P may either be trapped in the interface due to the re-oxidation of Fe (II) to Fe (III) (Gunnars & Blomqvist, 1997) or be mobilized to the overlying water. Although the ratio between Fe and P in water or oxalate extracts showed much lower

Akaike weights than P in water, extracts for the overall analysis of soils with water-extractable Fe: P > 5 all showed low P mobilization rates, below 0.067 mg m^{-2} d^{-1}, even when water-extractable P reached 8 μmol L^{-1} FW (data not shown). This is a strong indication that Fe plays an important role in P mobilization rates as long as the water layer is aerobic, similar to underwater soils (Geurts et al., 2011). This also means that if flooding water becomes anaerobic, for instance by the cover of floating-leaved plants (Van Kempen et al., 2012), or by the prolonged flooding of soils, resulting in oxygen depletion and increased microbial use of iron as an alternative electron acceptor (Lamers et al., 1998; Zak & Gelbrecht, 2007; Loeb et al., 2008) P mobilization rates may become much higher than those measured here because of the release of iron-bound (oxalate-extractable) P.

Next to Fe, S has also been shown to play a significant role in P mobilization from flooded soils. Under anaerobic conditions SO_4^{2-} is reduced to sulfide which has a much stronger affinity to Fe than P, leading to FeS_x precipitation (Sperber, 1958; Lamers et al., 2002b; Zak et al., 2006; Smolders et al., 2010). Consequently, Fe becomes less available for binding P, which can lead to an additional release of P (Smolders et al., 2006). In this study, the inclusion of S-related variables did not add to predictability (Table 2.1). However, this may change after prolonged flooding of soils subjected to high SO_4^{2-} loads (Lamers et al., 1998).

Implications for wetland creation

The present study shows that it is indeed possible to predict potential P release rates from a wide range of agricultural and former agricultural lands under aerobic surface water conditions during short-term flooding through the simple measurement of water-extractable P. This provides a valuable predictive tool for the selection of suitable locations for temporary water storage and retention. The temperature sensitivity results also show that eutrophication risks can be expected to be much higher in summer than in winter. Topsoil removal is a commonly used measure in nature restoration to lower the P availability in the top layer, given that the soil exposed after topsoil removal usually contains much less P. This also reduces the risk of nutrient release upon inundation, which will prevent water eutrophication during flooding (Lamers et al., 2006; Smolders et al., 2008; Emsens et al., 2015). However, our results show that prior to topsoil removal it is important to determine P concentrations along the soil profile to establish whether this is necessary, and how much soil should be excavated.

Conclusions

- For a wide range of soils, water-extractable P is the best predictor of P mobilization rates during temporary flooding; 86.9% of the variation in P mobilization rates could be explained by this variable. In addition, Fe also plays an important role in the process of P mobilization during short-term flooding.

- Release rates were found to be temperature sensitive. The average Q_{10} of soil P mobilization rates was 2.8, which can be included in predictions based on differences in seasonal hydrology, but its variation around the average could not be explained by any of the soil characteristics measured.
- Depth profiles provide information whether topsoil removal is a feasible or necessary option to prevent high P mobilization rates.
- Water-extractable P is shown to be a widely applicable, cost-effective and simple tool to predict P mobilization rates during short-term flooding, which can greatly assist water managers during their decision-making processes concerning the optimal location for the construction of water storage areas, the restoration of riparian wetlands, and the combinational use of different ecosystem services in climate-adaptive water and nature management.

Acknowledgements

We would like to thank Evi Verbaarschot, Gijs Van Dijk and Jose van Diggelen, Moni Poelen for their help to collect soils in the Netherlands and Ankie De Vries-Brock, Germa Verheggen, Paul Van der Ven, Roy Peters and Sebastian Krosse for their assistance with the chemical analyses. Yingying Tang is funded by China Scholarship Council (CSC), file number 201206140019.

Supplementary information

Supplementary Table 2.1 Coordinates of sampling locations in the Netherlands.

code	latitude	longitude	area	soil type
1	51°59′N	4°40′E	Zuidplas	peat
2	51°59′N	4°37′E		
3	51°59′N	4°38′E		
4	51°59′N	4°38′E		
5	51°59′N	4°39′E		
6	52°25′N	4°59′E	Burkmeer	
7	52°25′N	4°59′E		
8	52°25′N	4°59′E		
9	52°27′N	4°55′E	Ilperveld	
10	52°27′N	4°55′E		
11	52°26′N	4°55′E		
12	52°26′N	4°56′E		
13	52°31′N	4°48′E	Wormer-Jisperveld	
14	52°31′N	4°48′E		
15	52°31′N	4°49′E		
16	52°31′N	4°50′E		
17	52°07′N	6°28′E	Stelkampsveld	sand
18	52°06′N	6°28′E		
19	52°04′N	6°21′E	Hallerlaak	
20	52°04′N	6°21′E		
21	52°04′N	6°22′E		
22	52°04′N	6°22′E		
23	52°04′N	6°22′E		

Supplementary Table 2.2 Soil characteristics and P mobilization rates of peat and sand soils in the experiment. P_{water} represents water-extractable P; OM represents organic matter content.

Code	Depth	P_{water} (umol L^{-1} FW)	OM (g L^{-1} FW)	Fe/P_{total} (μmol μmol^{-1})	P_{olsen} (umol L^{-1} FW)	$Fe/P_{oxalate}$ (μmol μmol^{-1})	PO_4 mobilization rates (μmol m^{-2} d^{-1})	
							18°C	8°C
1	top	18.16	217.99	7.54	1159.54	10.89	5.42	n.a.
	deeper	9.02	223.27	9.72	736.94	18.30	1.32	0.53
2	top	35.80	229.75	3.03	2757.71	3.69	19.12	19.86

	deeper	5.56	176.63	4.92	980.99	5.72	8.27	4.14
3	top	5.15	116.67	10.10	2107.82	8.79	3.50	0.71
	deeper	4.19	146.31	15.92	967.56	12.21	1.12	1.21
4	top	3.569	197.12	6.04	1482.36	8.80	1.69	0.91
	deeper	4.33	188.67	6.63	1049.95	7.73	1.39	0.71
5	top	29.05	176.63	4.46	3055.34	3.29	16.29	8.37
	deeper	20.86	192.23	5.12	1684.54	4.43	7.13	2.61
6	top	71.43	249.15	3.46	1156.00	8.59	n.d.	n.d.
	deeper	80.67	162.25	3.54	298.60	15.33	37.68	21.13
7	top	6.86	215.12	8.97	1491.61	12.30	n.d.	n.d.
	deeper	6.45	168.56	10.53	304.00	20.97	1.60	0.80
8	top	2.02	241.07	7.99	426.05	21.77	0.94	0.77
	deeper	4.16	146.13	7.43	105.85	40.61	0.63	0.51
9	top	12.58	189.97	5.07	477.09	10.14	0.48	0.53
	deeper	1.02	126.11	15.22	78.31	35.60	1.34	0.55
10	top	2.74	186.87	5.27	275.18	20.77	1.36	1.54
	deeper	1.65	113.67	13.50	48.44	50.58	0.39	0.53
11	top	2.71	196.83	6.35	248.80	10.70	0.59	1.27
	deeper	2.12	81.79	3.45	30.59	30.14	0.48	1.10
12	top	3.25	157.32	8.18	72.30	35.45	0.55	0.43
	deeper	2.73	91.13	11.15	24.52	155.33	0.38	0.58
13	top	3.85	208.06	6.51	536.19	9.08	1.19	n.a.
	deeper	2.99	127.07	3.90	62.43	38.38	0.65	0.32
14	top	11.81	195.96	6.67	916.66	7.19	0.68	n.a.
	deeper	13.56	186.44	5.38	564.22	6.05	1.16	0.73
15	top	7.74	194.25	5.95	754.11	7.01	1.61	0.90
	deeper	3.88	132.69	9.96	90.76	15.73	0.55	0.77
16	top	4.14	173.01	7.77	700.23	9.65	1.02	0.03
	deeper	4.16	152.60	15.86	91.11	31.54	0.42	0.40
17	top	10.14	98.63	5.39	2027.72	6.93	3.46	0.37
	deeper	20.80	56.20	4.19	2305.54	2.86	6.79	3.96
18	top	4.80	62.92	7.59	3537.90	5.33	0.72	1.55
	deeper	3.00	25.80	31.17	1016.53	12.56	2.60	1.30
19	top	10.83	76.36	16.39	1907.76	5.86	3.09	2.30
	deeper	14.75	92.72	18.45	2077.57	5.87	3.68	1.51
20	top	17.91	59.10	17.99	2676.02	7.89	3.23	1.31
	deeper	9.35	37.30	18.79	1254.63	6.01	6.30	1.93
21	top	20.63	98.50	11.51	2413.87	5.01	7.24	3.05
	deeper	7.00	119.00	32.47	1291.99	13.98	1.82	1.02
22	top	258.15	77.04	1.06	4452.04	1.11	41.28	27.73
	deeper	269.46	49.85	0.75	4627.16	0.44	20.35	9.21
23	top	4.90	127.52	14.11	1134.34	16.20	1.11	0.60

deeper	1.22	35.31	14.06	925.02	10.44	1.70	0.44

Note that n.a. means not available; n.d. means not determined because soil became buoyant.

Chapter 3

Aquatic macrophytes can be used for wastewater polishing, but not for purification in constructed wetlands

Yingying Tang[§], Sarah F. Harpenslager[§], Monique M.L. van Kempen, Evi J.H. Verbaarschot,

Laury M.J.M Loeffen, Jan G.M. Roelofs, Alfons J.P. Smolders, Leon P.M. Lamers

[§]Y.T. and S.F.H. contributed equally to this study

Biogeosciences Discuss. (2016). doi:10.5194/bg-2016-80

Abstract

The sequestration of nutrients from surface waters by aquatic macrophytes and soils provides an important service of both natural and constructed wetlands. While emergent species take up nutrients from the soil, submerged and floating macrophytes filter nutrients directly from the surface water, which may be more efficient in constructed wetlands. It remains unclear, however, whether their efficiency is sufficient for wastewater purification, and how plant species and nutrient loading affects nutrient distribution over plants, water, and soil. We therefore determined nutrient removal efficiencies of different vegetation (*Azolla filiculoides*, *Ceratophyllum demersum* or *Myriophyllum spicatum*) and soil types (clay, peaty clay and peat) at three nutrient input rates, in a full factorial, outdoor mesocosm experiment. At low loading (0.43 mg P m^{-2} d^{-1}), plant uptake was the main pathway (100%) for phosphorus (P) removal, while soils showed a net P release. *A. filiculoides* and *M. spicatum* showed the highest biomass production and could be harvested regularly for nutrient recycling, whereas *C. demersum* was outcompeted by spontaneously developing macrophytes and algae. Higher nutrient loading only stimulated *A. filiculoides* growth. At higher rates ($\geq$ 21.4 mg P m^{-2} d^{-1}) 50–90% of added P ended up in soils, with peat soils becoming more easily saturated. For nitrogen (N), 45–90% was either taken up by the soil or lost to the atmosphere at loadings $\geq$ 62 mg N m^{-2} d^{-1}. This shows that aquatic macrophytes can indeed function as an efficient nutrient filter, but only for low loading rates (polishing), not for high rates (purification). The outcome of this controlled study not only contributes to our understanding of nutrient dynamics in constructed wetlands, but also shows the importance of wetland soil characteristics. Furthermore, the acquired knowledge may benefit the application of macrophyte harvesting to remove and recycle nutrients from both constructed wetlands and nutrient-loaded natural wetlands.

Introduction

Excess loading of phosphorus (P) and nitrogen (N) from domestic, agricultural and industrial wastewaters is the main cause of eutrophication of aquatic ecosystems, damaging their ecological quality and functioning (Kronvang et al., 2005; Kantawanichkul et al., 2009). Surface water eutrophication can lead to algal and cyanobacterial blooms, die-off of indigenous vegetation and serious decrease in biodiversity (Pretty et al., 2003; Conley et al., 2009). In recent decades, wetlands have been constructed to mitigate eutrophication of watercourses, lakes and seas by reducing the nutrient loads in discharge water of wastewater treatment plants, farmlands, households or industries (Brix & Arias, 2005; Mitsch et al., 2005).

Constructed wetland systems (CWS) use macrophytes (free surface flow systems) or a combination of macrophytes and soil (subsurface flow systems), to remove nutrients from the water (Lin et al., 2003). These systems are either used as stand-alone water purification systems (Vrhovšek et al., 1996; Jing et al., 2001) or as a polishing method of pre-treated wastewater (Kaseva, 2004; Greenway, 2005). The most commonly used macrophyte species are emergent genera such as *Typha, Phragmites, Scirpus, Phalaris* and *Iris* (Vymazal, 2011). Advantages of CWS include utilization of natural processes, low cost and energy requirements, and easy operation and maintenance (Brix, 1999; Konnerup et al., 2009). As a result of low maintenance, however, these systems easily become saturated, especially with P, and therefore only work efficiently for a limited amount of time (Drizo et al., 2002).

Although much research has focused on the optimal design of CWS with respect to the most efficient macrophyte species (Lin et al., 2002; Scholz & Xu, 2002), only few have investigated the possibility of using floating or submerged aquatic macrophytes in treatment systems. While helophytes mainly take up nutrients from the soil, floating and submerged aquatic macrophytes, such as *Azolla* spp. or *Myriophyllum* spp., can also take up nutrients from the water layer (Best & Mantai, 1978; Van Kempen et al., 2012). By regularly harvesting these plants, nutrients may be drained from the system. The aquatic biomass can then be used in various bio-based applications, for instance, as a bio-fertilizer or as fodder for livestock (Hauck, 1978).

There is a suite of mechanisms involved in the processes of nutrient removal and recovery in natural and constructed wetlands, including adsorption, precipitation, plant absorption, volatilization, and microbial processes such as iron oxidation, nitrification, DNRA (dissimilatory nitrate reduction to ammonium) and anammox (anaerobic ammonium oxidation) (Van Loosdrecht & Jetten, 1998; Van Dongen et al., 2001; Kadlec & Wallace, 2008; Wu et al., 2014). These mechanisms are generally affected by factors such as nutrient loading, plant species and soil characteristics (Gale et al., 1994; Tanner, 1996; Jampeetong et al., 2012). So far, most studies have focused on the effects of only one or two of these factors on nutrient retention in wetlands, whereas little information is available on interactions among plant species, soil type and nutrient loading. Only

by including all interactions, however, can nutrient sequestration efficiency of wetland plants and soils under different loads be assessed.

Here, we studied the effects of plant species, nutrient loading and soil type on nutrient uptake rates of aquatic macrophytes and nutrient retention rates of soils. Using a full-factorial outdoor mesocosm experiment, we studied the nutrient uptake rates of three different aquatic macrophytes, *Azolla filiculoides*, *Ceratophyllum demersum* and *Myriophyllum spicatum*, growing on peat, peaty clay or clay soils. Three different, environmentally relevant, nutrient loadings of P (0.43, 21.4 and 85.7 mg P m^{-2} d^{-1}) and N (1.3, 62 and 249 mg N m^{-2} d^{-1}) were applied to the mesocosms, representing pre-treated (low nutrient loading), and eutrophic and hypertrophic wastewater input (medium and high nutrient loading). By studying the resulting distribution of P and N among the different soil, macrophyte and water compartments, we aimed to determine whether nutrient removal by floating or submerged aquatic macrophytes may be an efficient approach for polishing or purifying wastewater.

Materials and methods

Experimental set-up

Twenty-seven mesocosms (185 cm Ø, 90 cm depth) were sunk into the ground outside the greenhouse facility at Radboud University (Nijmegen, The Netherlands). All mesocosms were filled with 20 cm (135 L) of clay (originating from Lalleweer, 53°16′N, 6°59′E; n = 9), peaty clay (originating from De Deelen, 53°01′N, 5°55′E; n = 9) or peat (originating from Ilperveld, 52°27′N, 4°56′E; n = 9), after which they received a layer of 50 cm of Nijmegen tap water. Soil characteristics are displayed in Table 3.1, expressed per unit volume to enable comparison among soil types with respect to nutrient exchange and plant nutrient availability. In all mesocosms, crossed transparent carbon fiber plates were used to create four fully isolated quarters. We did not include non-vegetated treatments because: 1) our focus was on complete ecosystems in constructed and natural wetlands, i.e. including soil and vegetation; 2) bare soils always show spontaneous vegetation development if light and nutrient conditions suffice (see section Plant measurements); 3) continuous plant removal would lead to significant soil disturbance; and 4) dark conditions would affect soil biogeochemistry. Mesocosms were randomly assigned to "low", "medium" or "high" nutrient loading treatment (n = 3 for all). To create these, treatment solutions were added three times a week to enable loading rates of 0.43, 21.4 and 85.7 mg P m^{-2} d^{-1} (added as NaH_2 $PO_4.H_2O$ and atmospheric deposition of 0.1 kg P ha^{-1} y^{-1}) (Furnas, 2003) and 1.3, 62 and 249 mg N m^{-2} d^{-1} (added as NH_4 NO_3 and atmospheric N deposition of 20 kg N ha^{-1} y^{-1} in this part of the Netherlands; TNO) (De Leeuw et al., 2001). In the results and discussion sections, treatments will be called 0.43 (low), 21.4 (medium) and 85.7 (high) mg P m^{-2} d^{-1}, according to their respective P loading.

Table 3.1. Soil characteristics of peat, peaty clay and clay soils used in the experiment (± SE; n = 36). pH and Total inorganic carbon (TIC) are derived from porewater analyses, whereas all other analyses were performed using fresh or dry soil (see Sect. Chemical analyses).

Soil	Bulk density (kg DW.L^{-1} FW)	Organic matter %	pH	TIC (µmol L^{-1})	Salt extractable N ($NO_3^- + NH_4^+$) (µmol L^{-1} FW)	Olsen-P (umol L^{-1} FW)	Total-P (mmol L^{-1} FW)	Total-Fe (mmol L^{-1} FW)	Total-Al (mmol L^{-1} FW)	Total-Ca (mmol L^{-1} FW)
Peat	0.15 (0.00)^{C}	43.73 (0.80)^{A}	7.20 (0.02)^{A}	8825.84 (120.36)^{A}	551.72 (58.71)^{B}	269.41 (13.16)^{B}	4.98 (0.19)^{B}	47.15 (0.92)^{B}	55.43 (1.80)^{B}	65.05 (1.06)^{B}
Peaty clay	0.23 (0.01)^{B}	34.39 (1.63)^{B}	6.92 (0.03)^{B}	5892.89 (240.56)^{B}	494.11 (70.17)^{B}	153.90 (13.98)^{C}	3.39 (0.19)^{C}	58.72 (4.32)^{B}	67.84 (5.37)^{B}	62.14 (5.02)^{B}
Clay	1.00 (0.01)^{A}	5.07 (0.24)^{C}	7.18 (0.04)^{A}	10189.53 (537.67)^{A}	1063.66 (123.98)^{A}	1104.48 (18.69)^{A}	22.25 (0.41)^{A}	402.74 (5.26)^{A}	438.77 (8.05)^{A}	101.85 (1.31)^{A}

Significant differences among soil types are indicated by different capital letters (A, B and C).

Plant measurements

In July 2013, environmentally relevant densities (based on personal field observations) of *Ceratophyllum demersum* (5.03 ± 0.24 g DW m^{-2}; rigid hornwort, submerged macrophyte), *Chara hispida* (8.66 ± 0.69 g DW m^{-2}; bristly stonewort, submerged macroalga) and *Myriophyllum spicatum* (5.31 ± 0.60 g DW m^{-2}; Eurasian water-milfoil, submerged macrophyte) were planted randomly in each of three quarters of every mesocosm to establish. In April 2014, patches of *Azolla filiculoides* (28.39 ± 0.88 g DW m^{-2}; water fern, floating macrophyte) were added to the water layer of the remaining quarter. Apart from these four introduced species, other species colonized the quarters, including *Zanichellia* spp. and floating algae. During the experimental period, 20% of the total plant biomass was harvested when vegetation reached 100% cover to avoid space limitation. During the final harvest, biomass of all present species was harvested separately and dried (48 h at 60°C), after which they were weighed, ground and homogenized. As *C. hispida* was completely outcompeted by spontaneously developing vegetation, the quarters with this species were excluded from the results.

Chemical analyses

Surface water samples were collected every week between May and October 2014, whereas pore water samples were collected anaerobically every month using ceramic soil moisture cups (SMS rhizons, Eijkelkamp, Giesbeek, Netherlands). pH of water samples was measured using a combined Ag/AgCl electrode (Orion, Thermo Fisher Scientific, Waltham, MA, U.S.A.) with a TIM840 pH meter (Radiometer Analytical, Lyon, France). Total inorganic carbon (TIC) of water samples was measured using an Infra-red Gas Analyzer (IRGA; ABB Analytical, Frankfurt, Germany). Concentrations of PO_4^{3-}, NO_3^- and NH_4^+ in the surface water and pore water were measured

colorimetrically on an Auto-Analyzer III system (Bran & Luebbe, Norderstedt, Germany) by using ammonium molybdate (Henriksen, 1965), hydrazine sulphate (Kamphake et al., 1967) and salicylate (Grasshoff & Johannsen, 1972), respectively. Concentrations of total Al, Fe, Ca, and P were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES; IRIS Intrepid II, Thermo Fisher Scientific, Franklin, MA, U.S.A.).

Soil samples were collected at the end of the experiment, and subsequently volume weighted and dried for 48 h at 60°C to determine bulk density. Dry soil samples were heated for 4 h at 550°C and re-weighed to determine organic matter content. Furthermore, 200 mg of dry soil was digested in a microwave oven (MLS-1200 Mega, Milestone Inc., Sorisole, Italy) with 4 mL 65% HNO_3 and 1 mL 30% H_2O_2, after which digestates were analyzed by ICP-OES (see above). Plant available P was determined by extraction according to Olsen et al. (1954), whereas an NaCl-extraction was performed to determine exchangeable N ions ($NO_3^- + NH_4^+$) as described in Tomassen et al. (2004). Total P concentrations in plants were determined by digestion of 200 mg of dry plant material and analyzed as described above. Furthermore, 3 mg of dry plant material was combusted to determine C and N content using an elemental analyzer (Carlo Erba NA 1500, Thermo Fisher Scientific, Waltham, MA, USA).

Budget calculations

For both N and P, nutrient budgets were calculated to determine the distribution among biomass, soil and other components. Cumulative biomass production and nutrient content of submerged or floating macrophytes (target species and others) were used to calculate plant uptake rates of N and P. Furthermore, nutrient changes in surface water and pore water were calculated from changes of N (NO_3^- and NH_4^+) and P concentrations (end minus start). After subtracting the N and P uptake of plants and water components from the external loading, we assume that the remainder is either stored in the soil or, in case of N, lost through denitrification or anammox.

Statistical Analyses

All analyses were performed using the software program R (version 3.2.1; R development Core Team, 2015). The effects were considered significant if $P < 0.05$. In order to meet the assumption that residuals fit a normal distribution and homogeneity of variance, we transformed soil characteristics, N (NO_3^- and NH_4^+) and P concentrations in surface water, biomass production rates, N: P ratios in macrophytes, N and P budgets and N and P sequestration rates (response variables) by log (response variable) or log (response variable + 1) in case the lowest value of a variable was below one. Linear mixed models were used to test the main effects and interactions of treatments on soil characteristics, biomass production rates, the ratios between N and P, N (NO_3^- and NH_4^+) and P concentrations in surface water (except for treatments also including time as a main effect in this model), and nutrient budgets with mesocosm number as a random

effect, by using R package nlme. Tukey tests were used to find differences between treatments by using R package multcomp. We analyzed the influence of nutrient loadings on P and N sequestration rates using linear and logistic regression models. All graphs were plotted using R package ggplot2.

Results

Surface water and pore water quality

Over time, surface water P and N ($NH_4^+ + NO_3^-$) concentrations increased (Figs. 3.1 and 3.2; $X^2 = 4.26$; $P < 0.05$ and $X^2 = 35.61$; $P < 0.000$ for P and N respectively), especially towards the end of the growing season. When macrophytes were growing on peat or peaty clay soils, P concentrations in the surface water increased with increasing external P loading ($X^2 = 115.87$; $P < 0.000$ and $X^2 = 88.94$; $P < 0.000$ for peat and peaty clay soils respectively).

Porewater nutrient concentrations depended on soil type. Peat soils had the highest P concentrations in the pore water, whereas the lowest were found in clay soils ($X^2 = 12.07$; $P < 0.01$; data not shown) even though their total P and Olsen P concentrations were much higher than for the other two soils (Table 3.1). In addition, mesocosms filled with peat soils had higher N concentrations in the pore water than those with peaty clay and clay ($X^2 = 7.13$; $P < 0.05$; data not shown). Surface water and porewater together never contained more than 12% of total P and N added to the system at P loadings ≥ 21.4 mg P m^{-2} d^{-1} (Figs. 3.4 and 3.5).

Macrophyte productivity and nutrient ratio

Due to their high biomass production rates, *A. filiculoides* and *M. spicatum* could be harvested weekly and biweekly, respectively. *A. filiculoides* had the highest biomass production rates of all three macrophyte species ($X^2 = 55.45$, $P < 0.000$), whereas *C. demersum* grew best on peaty clay soils ($X^2 = 10.67$, $P < 0.01$), but almost disappeared when growing on clay and peat soils due to competition with algae and other non-target species (Fig. 3.3). Biomass production rates of *A. filiculoides* were significantly higher at high nutrient loading than at low nutrient loading ($X^2 = 11.39$, $P < 0.01$), whereas no effect of nutrient loading was found for the other macrophytes. In quarters with *C. demersum* there was a higher production rate of non-target species than in quarters with *A. filiculoides* and *M. spicatum* ($X^2 = 6.28$, $P < 0.05$). *A. filiculoides* showed high N: P ratios (> 24 mol mol^{-1}) when grown at ≤ 21.4 mg P m^{-2} y^{-1} ($P < 0.000$), whereas all other species generally showed N: P ratios ranging from 8 to 17 mol mol^{-1}, without an effect of soil type (Table 3.2).

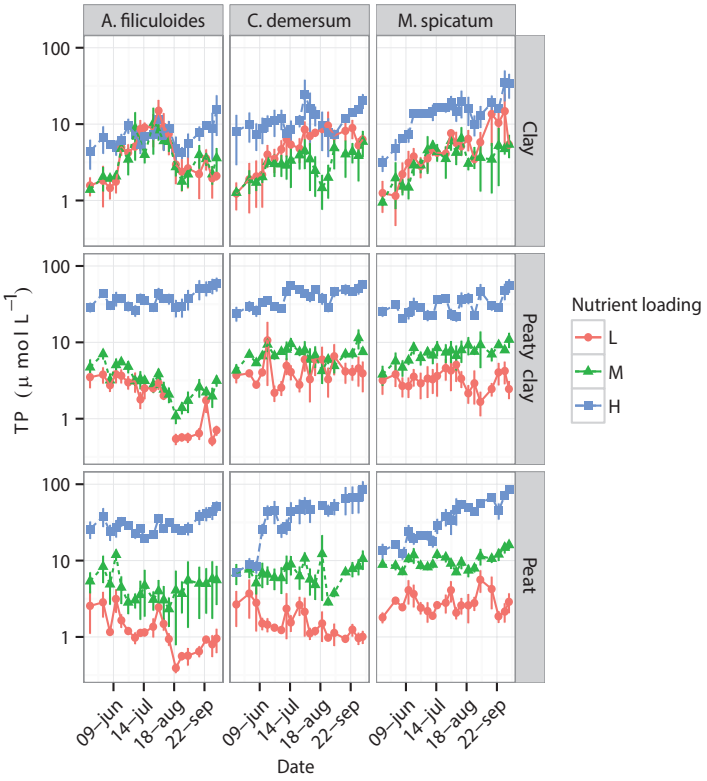


Figure 3.1. Surface water TP concentrations subjected to different nutrient loadings (L = 0.43 mg P m^{-2} d^{-1}; M = 21.4 mg P m^{-2} d^{-1}; H = 85.7 mg P m^{-2} d^{-1}) in mesocosms with different plant species (vertical panels) on clay, peaty clay or peat soils (horizontal panels) during the experiment. Average TP concentrations are given with SEM. Note the $\log_{10}$ scale for the y-axis.

Plant nutrient uptake

A. filiculoides and *M. spicatum* accumulated much more P than *C. demersum* ($X^2 = 23.66$, $P < 0.000$; Fig. 3.4). At a P loading of 0.43 mg m^{-2} d^{-1} around 100% of added P and N were accumulated by the targeted macrophytes (Figs. 3.4 and 3.5). For the quarters with *A. filiculoides* and *M. spicatum*, around 20–40% and 10–20% of the P added was taken up by target species at P loadings of 21.4 and 85.7 mg m^{-2} d^{-1}, respectively, regardless of soil types. *C. demersum*, on the other hand, never took up more than 20% of the P added at these loadings. Still, at a loading of 85.7 mg P m^{-2} d^{-1}, removal rates by macrophytes were significantly higher than at 0.43 mg P m^{-2} d^{-1} ($X^2 = 7.22$, $P < 0.05$; Fig. 3.4). The average P sequestration rates by *A. filiculoides* and *M. spicatum* were 3 to 9 mg m^{-2} d^{-1} at P loadings $\leq$ 21.4 mg m^{-2} d^{-1}. At a high P loading of 85.7 mg m^{-2} d^{-1}, the average P removal rates by *A. filiculoides* and *M. spicatum* were 16 to 20 and 6 to 14 mg m^{-2} d^{-1}, respectively. In addition, *C. demersum* had higher P and N uptake rates

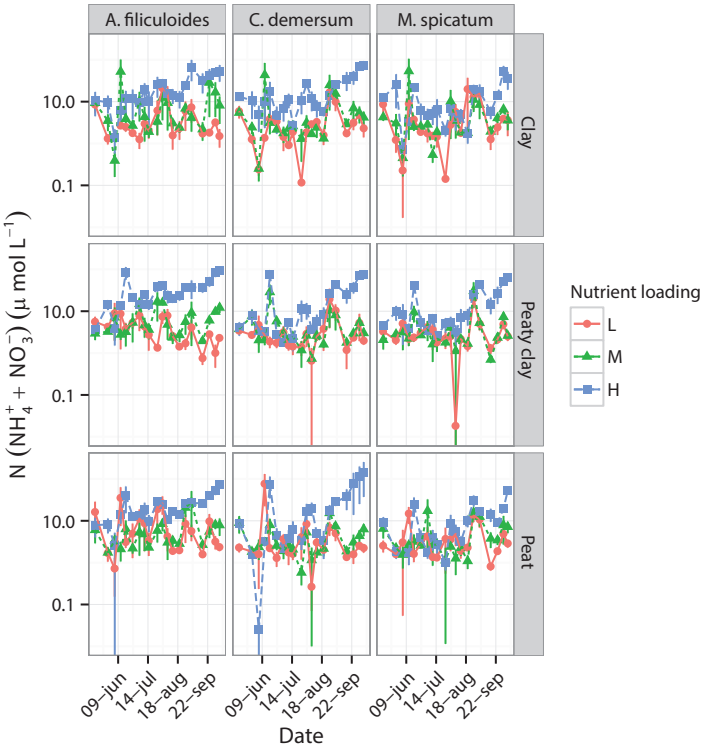


Figure 3.2. Surface water N ($NH_4^+ + NO_3^-$) concentrations subjected to different nutrient loadings (L = 0.43 mg P m^{-2} d^{-1}; M = 21.4 mg P m^{-2} d^{-1}; H = 85.7 mg P m^{-2} d^{-1}) in mesocosms with different plant species (vertical panels) on clay, peaty clay or peat soils (horizontal panels) during the experiment. Average N ($NH_4^+ + NO_3^-$) concentrations are given with SEM. Note the $\log_{10}$ scale for the *y*-axis.

in mesocosms with peaty clay compared to mesocosms with clay ($X^2 = 10.50$, $P < 0.01$; $X^2 = 10.43$, $P < 0.01$). In quarters with *C. demersum*, more P was taken up by other, spontaneously developing species than in quarters with *A. filiculoides* and *M. spicatum* ($X^2 = 6.89$, $P < 0.05$). In addition, these non-target plants in *C. demersum* quarters had lower P uptake rates on peaty clay than on peat and clay soils ($X^2 = 6.92$, $P < 0.05$). *A. filiculoides* and *M. spicatum* absorbed much more N than *C. demersum* and the final biomass of *A. filiculoides* had the highest N content (including N_2 fixed) among all macrophyte species ($X^2 = 10.28$, $P < 0.01$; Fig. 3.5). At high N loadings, less than 21 % of added N was removed by the targeted macrophytes.

Mobilization and adsorption of nutrients by the soil

At a P loading of 0.43 mg m^{-2} d^{-1}, soils were sources of P, whereas soils became P sinks at P loading $\geq$ 21.4 mg m^{-2} d^{-1} (Fig. 3.4). On average, 50 to 80% and 70 to 90% of P added accumulated in soils at medium and high nutrient loadings, respectively (Fig. 3.4). In quarters

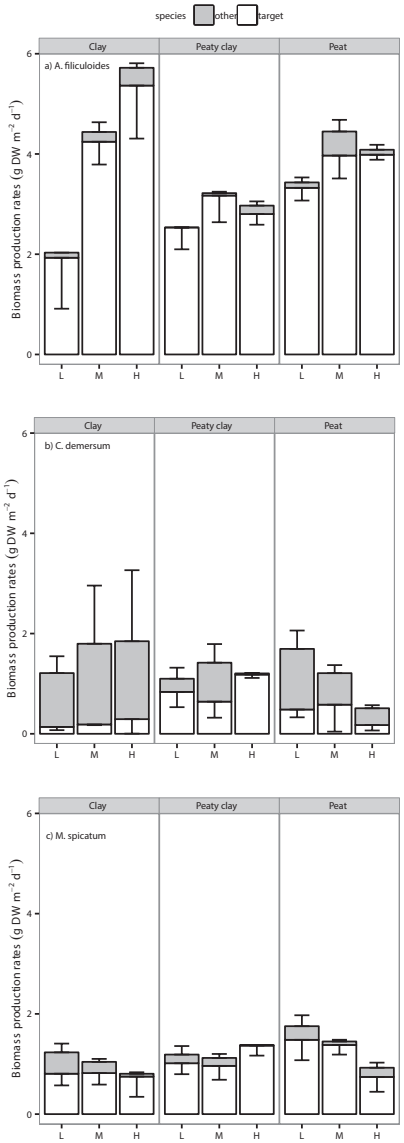


Figure 3.3. Biomass production rates (in g DW m^{-2} d^{-1}) of *A. filiculoides* (a), *C. demersum* (b), *M. spicatum* (c) and other, non-target plants (e.g. floating algae, *Zanichellia* spp and other plants) grown on different soil types and subjected to different nutrient loadings (L = 0.43 mg P m^{-2} d^{-1}; M = 21.4 mg P m^{-2} d^{-1}; H = 85.7 mg P m^{-2} d^{-1}). Average biomass production rates of target species (-SEM) and other plants (+ SEM) are given.

with *C. demersum*, more P accumulated in the soil than in quarters with *A. filiculoides* ($X^2 = 11.25$, $P < 0.01$). As P loading increased, more P accumulated in the soils ($X^2 = 566.40$, $P < 0.000$). At medium and high N loads, 45 to 90% and 80 to 90%, respectively, was either taken up by the soil or lost to the atmosphere through denitrification/anammox.

Table 3.2. Plant tissue ratios between N and P for different macrophytes subjected to different nutrient loadings (0.43, 21.4 and 85.7 mg P m^{-2} d^{-1}) at the end of the experiment. Average N: P ratios of target species are given with standard error.

Species	Soil type	N: P (mol: mol)		
		0.43	21.4	85.7
A. filiculoides	Clay	34.77 (± 1.03)[a]	42.88 (± 4.12)[a]	17.87 (± 1.28)[b]
	Peaty clay	49.21 (± 3.66)[a]	24.10 (± 0.64)[b]	11.23 (± 0.32)[c]
	Peat	41.94 (± 0.23)[a]	24.17 (± 1.95)[b]	12.84 (± 0.75)[c]
C. demersum	Clay	8.92 (± 1.36)	9.16 (± 1.12)	NA
	Peaty clay	9.33 (± 0.97)	9.04 (± 1.59)	8.04 (± 0.84)
	Peat	16.95 (± 4.29)[a]	9.43 (± 0.69)[ab]	7.52 (± 0.93)[b]
M. spicatum	Clay	10.43 (± 1.39)	9.80 (± 0.53)	9.22 (± 1.92)
	Peaty clay	13.31 (± 1.80)[a]	10.24 (± 0.56)[ab]	8.40 (± 0.74)[b]
	Peat	10.14 (± 1.18)	9.66 (± 0.38)	8.34 (± 0.78)

Significant differences among different nutrient loadings are indicted by different lower case letters (a, b and c); there were no significant differences among soil types. Note that NA means that there were no replicates for this treatment.

Discussion

In our mesocosm experiment, we show that at low nutrient input ($\leq$ 0.43 mg P m^{-2} d^{-1}), 100% of external loading could be removed through macrophyte uptake, whereas with loadings $\geq$ 21.4 mg P m^{-2} d^{-1}, 50 to 90% of added P ended up in soils. Differences exist, however, between binding abilities of soils, with clay soils being able to immobilise P better than peaty clay or peat soils. Apart from P, macrophytes were able to remove no more than 65% and 21% of added N at loadings of 62 mg m^{-2} d^{-1} and 249 mg m^{-2} d^{-1}, respectively, while the remaining N was either stored in the soil or lost to the atmosphere through denitrification and/or anammox.

Growth and nutrient uptake of macrophyte species in constructed wetlands

With average biomass production rates of 3.4 and 1.0 g DW m^{-2} d^{-1}, respectively, *A. filiculoides* and *M. spicatum* showed the highest growth rates and therefore the best potential for being used to remove nutrients in constructed wetlands. Due to their high growth rates, these species could be harvested biweekly or even weekly. *C. demersum*, on the other hand, appeared to be less suitable, since this species was readily outcompeted by other species, such as floating algae and *Zanichellia spp*. P was removed most efficiently by *A. filiculoides*, followed by *M. spicatum and C. demersum*. Although a high P load (85.7 mg m^{-2} d^{-1}) resulted in increased uptake rates of 6 to 14 and

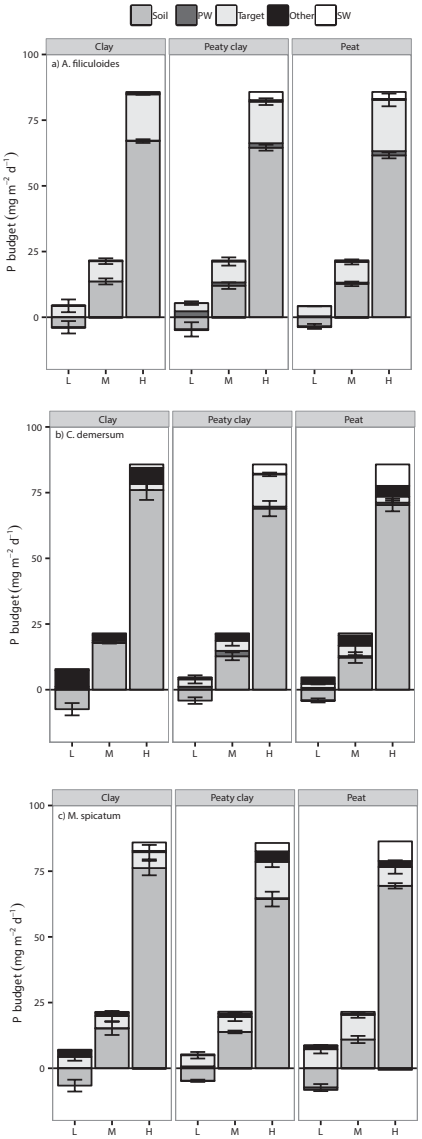


Figure 3.4. P budgets of soil, surface water, pore water, target species and other plants subjected to different nutrient loadings (L = 0.43 mg P m^{-2} d^{-1}; M = 21.4 mg P m^{-2} d^{-1}; H = 85.7 mg P m^{-2} d^{-1}) for (a) *A. filiculoides*, (b) *C. demersum*, and (c) *M. spicatum*. Standard errors are given only for soil and target species. PW = pore water, SW = surface water. Positive values represent P accumulation in relative parts; negative values represent P release from respective compartments.

even 16 to 20 mg P m^{-2} d^{-1} for *M. spicatum* and *A. filiculoides*, respectively, these rates were not sufficient to efficiently filter all added P from the system.

For *C. demersum*, nutrient sequestration rates increased linearly with increased nutrient loading,

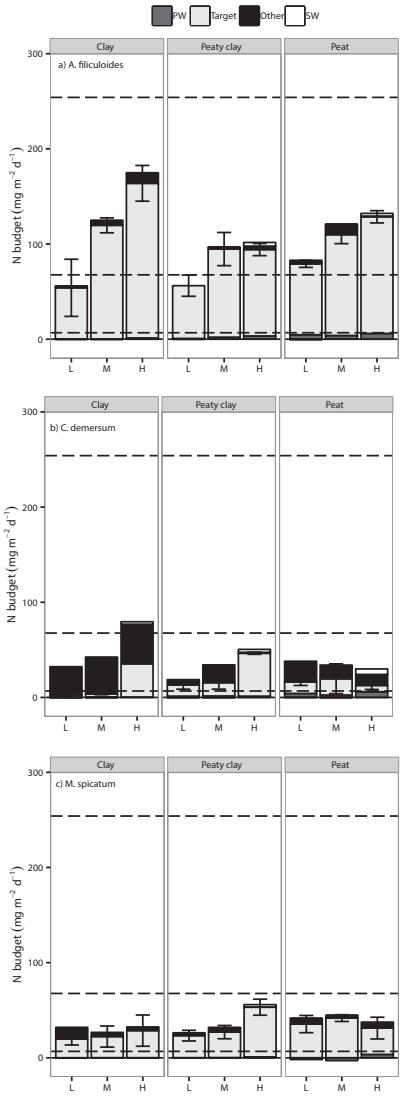


Figure 3.5. N distribution in surface water, pore water, target species and other plants subjected to different nutrient loadings (L = 0.43 mg P m^{-2} d^{-1}; M = 21.4 mg P m^{-2} d^{-1}; H = 85.7 mg P m^{-2} d^{-1}) from (a) *A. filiculoides*, (b) *C. demersum* and (c) *M. spicatum* macrophyte systems. Standard errors are given only for target plants. PW = pore water, SW = surface water. Positive values represent N accumulation in relative parts; negative values represent N release from respective compartments. The lowest, medium and highest dashed lines represent external N input at low, medium and high N loadings (including actual atmospheric N deposition), respectively.

while for *M. spicatum* there was a logistic response to external nutrient loading (Fig. 3.6). *A. filiculoides* showed linearly increasing P sequestration rates upon increased P loading and a logistic response to external N loading. These different response types between species most likely resulted from differences in main nutrient sources and nutrient limitation. For rooted *M. spicatum*, plants

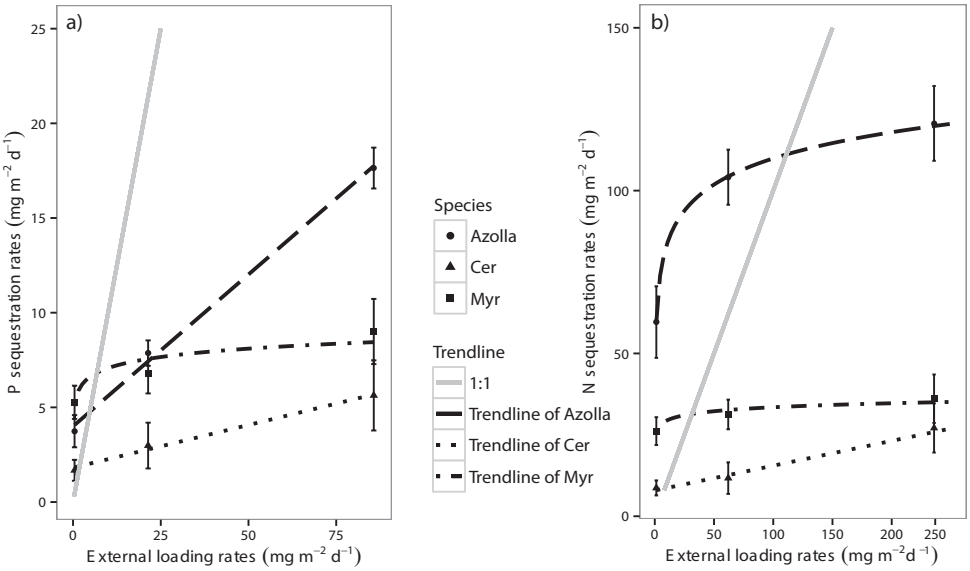


Figure 3.6. The correlations between external loading and nutrient sequestration rates of P (a) and N (b) by three different aquatic plant species. Standard errors and 1:1 line are given. Note that for *A. filiculoides* N_2 fixation is included in the sequestration rates, overestimating the effects of loading.

mainly rely on soil uptake (Best & Mantai, 1978; Barko & Smart, 1980; Carignan & Kalff, 1980), whereas for non-rooted *A. filiculoides* and *C. demersum* water is the main nutrient source (Denny, 1987; Mjelde & Faafeng, 1997). Our results indicate that at loads $\leq$ 22 mg P m^{-2} d^{-1}, *M. spicatum* is the most efficient P remover, whereas at loads $\geq$ 22 mg P m^{-2} d^{-1}, *A. filiculoides* is more efficient (Fig. 3.6a). In addition, the effective thresholds for P purification (100% removal) of *C. demersum, A. filiculoides*, and *M. spicatum* are 1.9, 4.8 and 6.8 mg P m^{-2} d^{-1}, respectively (Fig 3.6a). Threshold values for complete N removal are 8.6 and 31.4 mg N m^{-2} d^{-1} for *C. demersum* and *M. spicatum*, respectively (Fig. 3.6b). *A. filiculoides*, on the other hand, hardly ever becomes N limited due to its symbiosis with a diazotrophic microbial community (Handley & Raven, 1992). Under low external P loadings, *A. filiculoides* therefore displayed very high N: P ratios indicating P limitation at P loadings $\leq$ 21.4 mg P m^{-2} d^{-1}. *C. demersum*, on the other hand, having no access to soil or atmospheric N, probably showed N limitation in these systems, as indicated by their low N: P ratios. For all species, N: P ratios decreased with increasing P load.

Using aquatic macrophytes for polishing of pre-treated wastewater

Due to regular harvesting of *A. filiculoides* and *M. spicatum*, P and N were removed at rates of around 3 to 9 mg P m^{-2} d^{-1} and 31 mg N m^{-2} d^{-1} at loadings of 0.43 mg P m^{-2} d^{-1} and 1.3 mg N m^{-2} d^{-1}. These results are comparable to those found by Van Kempen (2013) who

found uptake rates of 3.7 mg P m^{-2} d^{-1} (13.4 kg ha^{-1} $year^{-1}$) and 13.7 mg N m^{-2} d^{-1} (50 kg ha^{-1} $year^{-1}$) in summer, and 4.8 mg P m^{-2} d^{-1} (17.5 kg ha^{-1} $year^{-1}$) and 69.3 mg N m^{-2} d^{-1} (253 kg ha^{-1} $year^{-1}$) in early fall for *A. filiculoides* grown in N-free water with 25 µmol L^{-1} PO_4. For *M. spicatum*, our results are in the same range as those reported by Smith and Adams (1986) and N uptake rates of 0.05–1.26 g N m^{-2} d^{-1} by *Myriophyllum aquaticum* reported by Nuttall (1985). Due to lowering of the O_2 concentration in the water layer, similar to other floating or densely growing submerged macrophytes (Caraco et al., 2006), these plants not only take up all P being discharged into the system, but additionally mobilize and take up P from the soil by their roots and the creation of anaerobic conditions (Wetzel, 2001).

Since uptake of nutrients by aquatic macrophytes depends on their biomass production and thus on macrophyte photosynthesis, these systems would only function optimally during the growing season. Under low external loading, soils will take up most of the P during winter, which can subsequently be mobilised and taken up by macrophytes in summer, creating an efficient and sustainable constructed wetland for water polishing in temperate climates.

Using aquatic macrophytes for wastewater purification

When P loading in the treatment water increases, uptake rates of *A. filiculoides* double or even triple, to rates around 6-24 mg P m^{-2} d^{-1}. The highest value is comparable to results of Reddy and DeBusk (1985), who reported P uptake rates of 43 $\pm$ 15 mg P m^{-2} d^{-1} by *A. filiculoides* grown in an N-free, 3 mg L^{-1} PO_4^{3-}-medium. Although plants could not take up all P at medium or high external P loadings, overall surface water quality remained around or below 12 µmol L^{-1} when clay sediments were used for the construction of the wetland. At the end of the growing season, however, plant uptake decreased and P availability in surface waters above peaty clay and peat soils increased strongly to concentrations around 60 and 72 µmol P L^{-1}, respectively, indicating not only inactivity of aquatic macrophytes but probably also P saturation of soils. Due to the 7–8 times higher Fe and Al contents (400 vs. 50–60 mmol L^{-1} FW, 450 vs. 60-70 mmol L^{-1} FW for Fe and Al, respectively) of clay soils, P was most probably immobilized more efficiently by clay (Reddy & DeLaune, 2008), which resulted in lower P concentrations in surface water above clay soils.

More than 98% of added N was removed from the surface water during the run of the experiment. As nutrient loading increased, the amount of added N that was removed by plant uptake decreased. Harvested biomass of target plants contained 31 mg N m^{-2} d^{-1} for *M. spicatum*, whereas in the quarters with *C. demersum*, non-target macrophytes or algae absorbed most N. For *A. filiculoides* it was difficult to calculate N removal rates due to unknown N_2 fixation rates leading to an overestimation of N uptake rates by *A. filiculoides*. N that was not taken up by plants, but was still removed from the water layer most likely ended up in the soil or was released to the atmosphere by denitrification and/or anammox (Hao et al., 2002; Van Der Star et al., 2007). On

average, inorganic N ($NH_4^+ + NO_3^-$) concentrations in the surface water were below 8 μmol L^{-1} with external loadings ≤ 62 mg N m^{-2} d^{-1} and around 20 μmol L^{-1} when receiving 249 mg N m^{-2} d^{-1}. At the end of the growing season, N concentrations increased under high nutrient loading, similar to P, suggesting nutrient leaching from senescing plants is more important than soil saturation.

Implications for management

We showed that in macrophytes-dominated CWS, submerged or floating macrophytes are able to remove most of the added nutrients at low P and N loadings, whereas at higher nutrient loadings, floating or submerged macrophytes can only remove 20–45% and 10–25% of the external P loads for 21.4 and 85.7 mg P m^{-2} d^{-1}, respectively. For water management, using fast growing aquatic macrophytes, such as *A. filliculoides* or *M. spicatum* regular mowing allows complete removal of added nutrients at relatively low nutrient loading (≤ 4.8 mg P m^{-2} d^{-1} or ≤ 6.8 mg P m^{-2} d^{-1}, respectively). Although *A. filiculoides* still extracted P and competed with soil adsorption at higher P loads (≥ 21.4 mg P m^{-2} d^{-1}), most external P ended up in the soil, eventually resulting in saturation. While aquatic macrophytes are able to remove this P from the soils by either creating anaerobic conditions or through root uptake, the external load will have to be reduced for this process to occur efficiently. Consequently, at these higher P and N loads, the macrophyte stage can only be used as an additional polishing step after a major part of the nutrients have been removed by other ways of water treatment.

Conclusions

Here, we show that aquatic macrophytes can be used for polishing, but not as a stand-alone purification treatment for nutrient removal from wastewater. At loads ≤ 22 mg P m^{-2} d^{-1}, *M. spicatum* is the best option, whereas at loads ≥ 22 mg P m^{-2} d^{-1}, *A. filiculoides* removes P more efficiently. Furthermore, we have shown that soil type is a previously underestimated factor influencing the efficiency of nutrient removal and immobilization. Especially at higher P loads, soils form highly important sinks and the saturation potential of the soil is therefore important. Clay soils should be preferred, as these take longer to become saturated than more organic soils.

Acknowledgements

The authors would like to thank Moni Poelen and Peter Cruijsen for their practical assistance and Ankie De Vries-Brock, Germa Verheggen, Jelle Eygensteyn, Paul Van der Ven, Roy Peters and Sebastian Krosse for their assistance with the chemical analyses. SFH, MMLvK and LMJML were funded by the European Union, GO EFRO 2007–2013 (Water-Rijk, Rich Water World). YT was funded by the China Scholarship Council (CSC; file number 201206140019).

Chapter 4

Rooting and plant density strongly determine greenhouse gas budget of water hyacinth (*Eichhornia crassipes*) mats

Ernandes Sobreira Oliveira Junior[§], Yingying Tang[§], Sanne J.P. van den Berg,

Leon P. M. Lamers, Sarian Kosten

[§] E.S.O.J. and Y.T. contributed equally to this study

Biogeosciences Discuss. (2016). doi:10.5194/bg-2016-297

Abstract

Water hyacinth occurs in numerous tropical and subtropical countries, either as a native or as an invasive exotic species, where it can establish large and dense mats. The plant is also frequently used for water purification and bioremediation purposes. Although it is a free-floating species, the plant roots into the sediment of shallow waters, tapping into the sediment nutrient pool. Its long and extensive root system strongly increases nutrient absorption, resulting in high growth rates and concurring high carbon sequestration rates. On the other hand, the plants may also fuel methane (CH_4) production as dense mats may deplete oxygen in the surface water and sediment below, which in combination with the high production of organic matter creates favorable conditions for methanogenesis. We hypothesize that water hyacinth vegetation acts as a strong greenhouse gas (GHG) sink due to its high growth rates, especially when (sediment) nutrient availability is high. Still, this sink may be counterbalanced by CH_4 release, which will be most pronounced when the plants are rooting in the sediment due to potential CH_4 shuttling from the sediment through the roots and leaves into the atmosphere (chimney effect). To mechanistically unravel the influence of water hyacinth on nutrient dynamics and greenhouse gas fluxes, we performed an aquarium experiment in which plant density and root access to the sediment were manipulated. Although plant cover led to lower concentrations of dissolved total phosphorus (DTP) and phosphate, there were no effects of density or rooting. We found no vegetation effect on the ebullition of CH_4, but its diffusion was 4.5 times higher at high plant coverage. Rooting increased CH_4 diffusion by 1.3 (high density) and 4 times (low density), demonstrating the chimney effect that we hypothesized. Independent of rooting, however, water hyacinth at high density sequestrated less carbon compared to low density, possibly due to space limited growth and self-shading. Overall, water hyacinth enhanced CH_4 emissions, especially when rooted. Due to water hyacinth's high CO_2 sequestration rates, the overall GHG budget in terms of CO_2 equivalents still resulted in water hyacinth mats being near-neutral or even a GHG sink, depending on water hyacinth density. Our results show that the effect of water hyacinth mats on GHG fluxes strongly depends on both plant density and contact with the sediment. This indicates that, when making regional GHG balances, not only plant presence but also its density and water depth – regulating sediment-root contact – should be taken into account.

Introduction

Water hyacinth (*Eichhornia crassipes*) is notorious worldwide because of the problems it poses to economy, society and ecology when occurring at high densities (Malik, 2007; Villamagna & Murphy, 2010). Its high tolerance range for environmental conditions including pH, temperature and nutrients (Gutiérrez et al., 2001; Wilson et al., 2005) provides an ample spectrum of colonization, and explains its wide-spread occurrence around the world. Its fast growth rates and rapid dispersal through asexual reproduction explains its ability to form large floating mats comprising high biomass (Pinto-Coelho & Greco, 1999).

The search for useful end products for this large amount of biomass has resulted in its appliance as a source for bioenergy production (Chanakya et al., 1993), fodder (Ding et al., 2001), biofertilizer (Zhao et al., 2012), and paper fiber (Reddy & Tucker, 1983). Water hyacinth is also frequently used for water purification and bioremediation purposes because of its high nutrient uptake rates (Aoyama & Nishizaki, 1993; Mandi, 1994; Polprasert & Khatiwada, 1998). Nutrient availability strongly determines *Eicchornia*'s growth rate as well as nutrient allocation (Xie et al., 2004). Maximum nutrient uptake efficiency is typically reached in the early growth stage (Reddy et al., 1989; 1990), explaining the formation of big mats in a few days (Téllez et al., 2008).

Water hyacinth's high growth rate results in high carbon dioxide (CO_2) uptake at rates of 3.4–5.4 g C-CO_2 m^{-2} day^{-1} as reported for tropical lakes (Peixoto et al., 2016). In these lakes, the vegetation even sets off open water CO_2 emissions, turning the system into CO_2 sink. While water hyacinth growth will decrease CO_2 emissions, its presence may simultaneously increase the emission of methane (CH_4) (Banik et al., 1993), having a global warming potential (GWP) of 34 times CO_2 over a 100 year time scale (Myhre et al., 2013). Therefore, even relatively low rates of CH_4 emissions could offset the high CO_2 assimilation, turning water hyacinth mats into a greenhouse gas (GHG) source. The high density of water hyacinth suppresses light penetration and therefore photosynthetic activity in the water below. In combination with reduced O_2 diffusion from the atmosphere into the water by its cover, this can result in anaerobic conditions below the plant mat (Reddy & DeBusk, 1991). Research performed in ditches and tanks showed that the combination of decreasing O_2 concentrations and high organic matter production by water hyacinth favors CH_4 emission. This effect was strongest after multiple years, probably due to organic matter accumulation (Banik et al., 1993).

Aquatic plants rooting in the sediment tend to enhance CH_4 emissions by transporting CH_4 directly from the sediment to the atmosphere (Bastviken et al., 2008; Bastviken, 2009), a process referred to as the chimney effect. Although water hyacinth is generally reported as a floating plant, the plant can root in the sediment when the water level is sufficiently low (less than 50 cm; personal observation), potentially increasing CH_4 emissions. This enlarged GHG effect may, however, be counterbalanced by enhanced growth rates and therefore CO_2 uptake rates, due to

increased nutrient uptake from both sediment and water.

All in all, the effects of water hyacinth mats on GHG emissions are therefore not at all straightforward. Only few studies have investigated the effects of water hyacinth on total GHG emissions (CH_4 and CO_2) (Banik et al., 1993; Attermeyer et al., 2016; Peixoto et al., 2016), and none have included the effects of plant density or rooting. Moreover, the few studies that investigated the effect of water hyacinth on GHG balance showed contrasting results (enhanced CH_4 emissions: Banik et al. (1993); decreased CH_4 emissions: Attermeyer et al. (2016)). We hypothesize that the differences found may be due to variation in density and whether or not the plants are rooted in the sediment. We therefore used a full-factorial, controlled indoor aquarium experiment aiming to elucidate the effects of plant density and sediment rooting on the nutrient dynamics and GHG fluxes of water hyacinth vegetation.

Materials and methods

Experimental set-up

The experiment was conducted in 24 glass aquaria of 24 L (20 × 20 × 60 cm; l × w × h) filled with a layer of 7 cm of fresh sediment, and a layer of 38 cm of demineralized water. The sediment was collected from a eutrophic drainage ditch (Ede, The Netherlands; 51°59′43.58″N, 5°38′38.91″L) in September 2014, and was sieved with a 5.0 mm sieve to remove stones and vegetation remnants. Sediment characteristics were determined at the beginning of the experiment (Table 4.1). The aquaria were placed in a water bath at 23°C in the greenhouse facilities of the Radboud University (Nijmegen, The Netherlands). A light frame of 220 μmol m^{-2} s^{-1} PAR (16h light/8h dark) was provided by Philips Green Power 400V/1000 WE lamps in a New E-Papillon 1000 W armature, to provide sufficient light in case of cloudy conditions.

Water hyacinth was collected from a commercial breeder (Nijmegen, the Netherlands) and cultivated in the greenhouse for approximately 10 months prior to the experiment, on organic sediment to which slow-release phosphorus granules. The experiment lasted for 59 days, from October to December 2014. The aquaria were randomly assigned to controls without plants, low density (50% of water hyacinth coverage) or high density (100% coverage) (n = 8 for each treatment). In half of the treatments, a mesh (1.0 mm mesh size) was placed just above the sediment (n = 12) to prevent the plants from rooting in the sediment, dividing the plant treatments into rooted and non-rooted treatments. There were 4 controls without, and 4 with a mesh (jointly referred to as 'controls'). We added individual water hyacinths to each aquarium: 1) 160 g to the low density treatment with mesh (non-rooted – 50%nR) or without mesh (rooted – 50%R); and 2) 413 ± 2.63 g to the high density treatment with mesh (non-rooted – 100%nR) or without mesh (rooted – 100%R) (Fig. 4.1). To maintain the low coverage, water hyacinth was harvested partially at day 31 and 45.

Table 4.1. Sediment characteristics at the beginning of the experiment (mean ± SEM; n = 3). All analyses were performed using fresh or dry sediment (see section Chemical analyses).

Characteristics	Unit	Sediment
Organic matter content	%	3.32 ± 0.24
Total-P	µmol g^{-1} DW	15.65 ± 0.73
Olsen-P	µmol g^{-1} DW	0.85 ± 0.06
Salt extractable NH_4^+	µmol g^{-1} DW	0.25 ± 0.05
Salt extractable NO_3^-	µmol g^{-1} DW	0.02 ± 0.00
Total-Fe	µmol g^{-1} DW	88.13 ± 2.69
Total-Al	µmol g^{-1} DW	81.49 ± 2.20
Total-Ca	µmol g^{-1} DW	100.43 ± 3.83

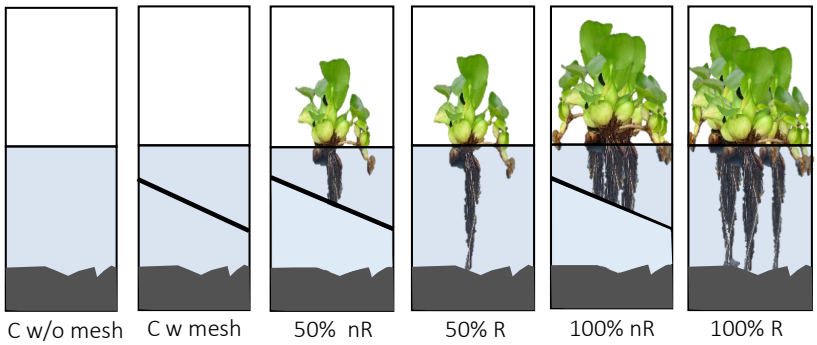


Figure 4.1. Experimental design. C w/o mesh represents control without mesh; C w mesh represents control with mesh; 50% nR represents low density with mesh; 50% R represents low density without mesh; 100% nR represents high density with mesh; 100% R represents high density without mesh.

Chemical analyses

Dissolved oxygen (DO), pH and temperature were measured weekly at both the surface and bottom of the water column, using a portable multi-meter (HQ40d multi, HACH, Loveland, Colorado, U.S.A.). Surface and porewater samples were collected anaerobically every week during the experiment using ceramic soil moisture samplers (SMS rhizons, Eijkelkamp, Giesbeek, Netherlands). Total inorganic carbon (TIC) of water samples was measured with an Infra-red Gas Analyzer (IRGA; ABB Analytical, Frankfurt, Germany). Concentrations of PO_4^{3-}, NO_3^- and NH_4^+ in the water samples were measured colorimetrically on an Auto-Analyzer 3 system (Bran & Luebbe, Norderstedt, Germany) by using ammonium molybdate (Henriksen, 1965), hydrazine sulphate (Kamphake et al., 1967) and salicylate (Grasshoff & Johannsen, 1972), respectively. Concentrations of dissolved total P (DTP) were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES; IRIS Intrepid II, Thermo Fisher Scientific, Franklin,

MA, U.S.A.). Dissolved organic carbon (DOC) in water samples was measured with a TOC-L CPH/CPN analyzer (Shimadzu, Kyoto, Japan) at the end of the experiment.

Sediment samples were collected at the start and end of the experiment, and subsequently dried for 48h at 60°C. Dry samples were heated for 4 hours at 550°C and re-weighed to determine organic matter content. Dried sediment (200 mg) was digested in a microwave oven (MLS-1200 Mega, Milestone Inc., Sorisole, Italy) using 4 ml 65% HNO_3 and 1 ml 30% H_2O_2 to determine total sediment Fe, Al, Ca and P concentrations. Digested solutions were analyzed by ICP-OES (see above). Olsen P extracts (plant available P) was determined by extraction according to Olsen et al. (1954), whereas a NaCl-extraction (exchangeable NH_4^+ and NO_3^-) was performed as described by Tomassen et al. (2004).

Greenhouse gas flux measurements

Diffusive flux

After 30, 38 and 45 days greenhouse gas (CO_2 and CH_4) diffusive fluxes were measured during the day and night using a lid on top of the aquarium to establish a closed system connected to a Picarro G2508 Greenhouse Gas Analyzer (Picarro Inc., Santa Clara, CA, USA). The lid was sealed air-tight with paste (Terostat IX, Teroson GmbH, Heidelberg, Germany).

Ebullitive flux

Total CH_4 fluxes (ebullitive + diffusive) were measured 3 times (on day 31, 39 and 46) during a period of 24 hours. During this time the glass lid (equipped with a rubber septum) was closed as described before. The increase in CH_4 concentration during 24 hours was determined by sampling the headspace (in duplicate) through the septum at the start and the end of the incubation and subsequent analyses on a gas chromatograph (HP 5890 equipped with a Porapak Q column (80/100 mesh), a flame ionization detector (GC-FID, Hewlett Packard, USA) and oven temperature 120°C). The total amount of CH_4 emitted was calculated by multiplying the change in CH_4 concentration in the headspace between $t = 0$ and at the end with the volume of the headspace. The ebullitive fluxes were calculated by subtracting diffusive CH_4 fluxes determined the day before from the total amount of CH_4 emitted.

Global Warming Potential

To evaluate the net GHG effect we used a global warming potentials (GWPs) of 34 for CH_4 converting to CO_2-eq fluxes as described by (Myhre et al., 2013).

Plant measurements

At the start of the experiment four extra plants were dried (148.37 ± 13.18 g FW and 9.00 ± 0.94 g DW) and used to analyze initial nutrient contents. At the end of the experiment all plants were collected. Water hyacinths were divided into leaves, petioles, and roots. The fresh plant samples were weighed and dried for 48h at 60°C, after which they were weighed again, grinded and homogenized. Subsequently, 200 mg of dry plant material was grinded and digested to determine total P concentrations in plants as described for the chemical analysis of sediment. An additional 3 mg of dry plant samples was combusted to determine C and N content with an elemental analyzer (Carlo Erba NA 1500, Thermo Fisher Scientific, Waltham, MA, USA).

Statistical analyses

Shapiro-Wilk's test and Bartlett's test were conducted to test normality of residuals and equality of error variances, respectively. Non-normal or heteroscedastic data were log transformed to meet these two requisites. Linear mixed models were used to test the main effects and interactions of treatments on water characteristics, DO, GHG fluxes, GWPs and the ratios of C:N, C:P, and N:P in different plant tissues with the aquarium number as a random effect, by using R package *nlme*. Tukey tests were performed to find differences between treatments by using R package *multcomp*. The effects of treatments and differences between treatments were considered significant if $P < 0.05$. All statistical analyses were carried out using the software program R (version 3.2.1; R development Core Team, 2015). All graphs were plotted by using SigmaPlot (v.11 Systat Software Inc, 2008).

Results

Biogeochemistry in water column and sediment

Dissolved oxygen (DO) concentrations did not vary in time ($P > 0.05$ for all treatments; data not shown), and were below saturation value (8.3 mg L^{-1} at 22°C). Average DO concentrations in the water layer were significantly lower in aquaria with plants (3.5 ± 0.2 mg L^{-1}) compared to the control aquaria without plants (5.7 ± 0.4 mg L^{-1}, $X^2 = 9.20$, $P < 0.05$), without a density effect (Fig 4.2).

The treatments including water hyacinth had about 10–50% lower concentrations of DTP and phosphate (PO_4^{3-}) in the surface water compared to the controls ($X^2 = 79.82$, $P < 0.001$ and $X^2 = 84.03$, $P < 0.001$ for DTP and PO_4^{3-} respectively; Table 4.2). In addition, they had lower NO_3^- concentrations in the surface water ($X^2 = 69.38$, $P < 0.001$).

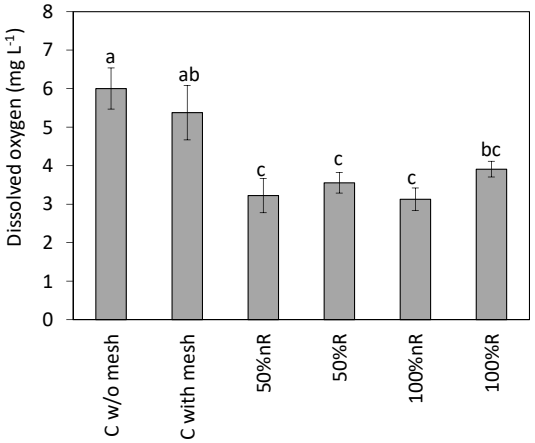


Figure 4.2. Mean dissolved oxygen concentrations (± SEM) of the water layer at 20 cm depth for controls (C), low density (50%), and high density (100%) of water hyacinth with (R) or without rooting (nR) in the sediment. Different lower case letters indicate significant differences between treatments by post hoc test ($P < 0.001$).

Table 4.2. Water characteristics in surface water during the experiment. All concentrations are given in μmol L^{-1}. DOC concentrations were determined at the end of the experiment (mean ± SEM; n = 4), whereas other parameters were analyzed multiple times during the experiment (overall average are given, mean ± SEM; n = 4).

Characteristics	C w/o mesh	C with mesh	50%nR	50%R	100%nR	100%R
DTP	17.4 ± 5.2^{a}	11.1 ± 1.4^{a}	2.2 ± 0.1^{b}	7.8 ± 4.6^{b}	6.4 ± 4.2^{b}	3.7 ± 1.3^{b}
PO_4^{3-}	12.8 ± 2.9^{a}	9.5 ± 1.4^{a}	1.6 ± 0.2^{b}	7.8 ± 5.4^{b}	1.4 ± 0.1^{b}	2.8 ± 1.0^{b}
NH_4^{+}	16.9 ± 6.2	6.2 ± 0.6	6.8 ± 0.5	60.6 ± 54.2	39.8 ± 33.8	6.2 ± 0.6
NO_3^{-}	9.5 ± 0.8^{a}	1.2 ± 0.6^{b}	1.8 ± 1.1^{b}	0.4 ± 0.1^{b}	0.6 ± 0.2^{b}	0.4 ± 0.2^{b}
TIC	734.3 ± 99.2^{b}	1020.4 ± 66.6^{a}	1052.0 ± 100.9^{a}	1417.0 ± 278.0^{a}	1319.0 ± 129.7^{a}	1212.0 ± 103.2^{a}
DOC	944.0 ± 135.9	890.7 ± 331.6	855.5 ± 198.0	469.3 ± 274.3	997.2 ± 273.6	629.0 ± 145.3

Significant differences among treatments are indicated by different lower case letters.

Nutrient concentrations in different plant tissues

Plant coverage or rooting did not show effects on nutrient concentrations in different plant tissues ($P > 0.05$). For low density, P concentrations in petioles were higher in rooted plants than in not-rooted plants ($P < 0.01$; See Supplementary Fig 4.1A). In addition, only low-density treatments showed higher P concentrations in petioles at the end of the experiment compared to the start ($P < 0.01$; See Supplementary Fig 4.1A). For all treatments P concentrations in roots were significantly higher at the end of the experiment than at the start of the experiment ($P < 0.001$; See Supplementary Fig 4.1B). Furthermore, only the high density rooted plants had higher N concentrations in petioles at the end of the experiment compared to the start ($P < 0.001$; See Supplementary Fig 4.2). N concentrations in petioles were higher in the treatment with high

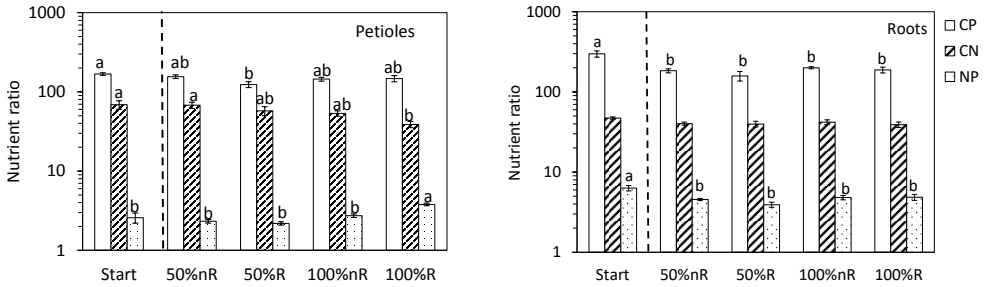


Figure 4.3. Ratios between C and P (white), between C and N (shaded), and between N and P (dotted) in petioles (left panel) and roots (right panel) of water hyacinth for low density (50%) and high density (100%) with (R) or without (nR) roots in the sediment at the end and start of the experiment. All nutrient ratios are given in mol mol^{-1}. Different lower case letters indicate significant differences between treatments including the start of the experiment ($P < 0.05$). Note the $\log_{10}$ scale on the *y*-axis.

density rooted plants than all other treatments ($P < 0.001$; See Supplementary Fig 4.2).

In general, nutrient did not change in time or differ between treatments. There were some exceptions however (Fig 4.3). Rooted plants growing at high density had a higher N: P ratio in the petioles (1.72, compared to 0.85) ($X^2 = 38.75$, $P < 0.001$), and the N: P ratio in the roots was lower at the end than at the start ($X^2 = 24.44$, $P < 0.001$).

Greenhouse gas fluxes

Total CH_4 fluxes were highest at high density (ANOVA for density effect; $X^2 = 8.72$, $P < 0.05$). This was mostly due to the high diffusion rates. At low density, diffusive CH_4 emissions were significantly higher in aquaria with rooted water hyacinth ($X^2 = 9.59$, $P < 0.01$) (Fig 4.4A). At high coverage the rooted water hyacinth tended to have higher diffusive CH_4 fluxes as well, albeit non-significant ($P = 0.90$). On average, CH_4 ebullition was 33.1 ± 28.7 mg CH_4 m^{-2} d^{-1} and did not significantly differ between the treatments (Fig 4.4A). In terms of CO_2, the controls without water hyacinth functioned as a source, whereas the treatments with water hyacinth functioned as a CO_2 sink ($X^2 = 17.08$, $P < 0.001$) (Fig 4.4B).

Discussion

We found that water hyacinth presence significantly increased diffusive CH_4 fluxes. These fluxes increased with plant density and, especially when plants were rooting in the sediment (Fig. 4.4A), stressing the role of water depth. Due to water hyacinth's high CO_2 sequestration rates, the overall GHG budget in terms of CO_2 equivalents still resulted in water hyacinth mats being near-neutral or even a GHG sink, depending on water hyacinth density (Fig. 4.5).

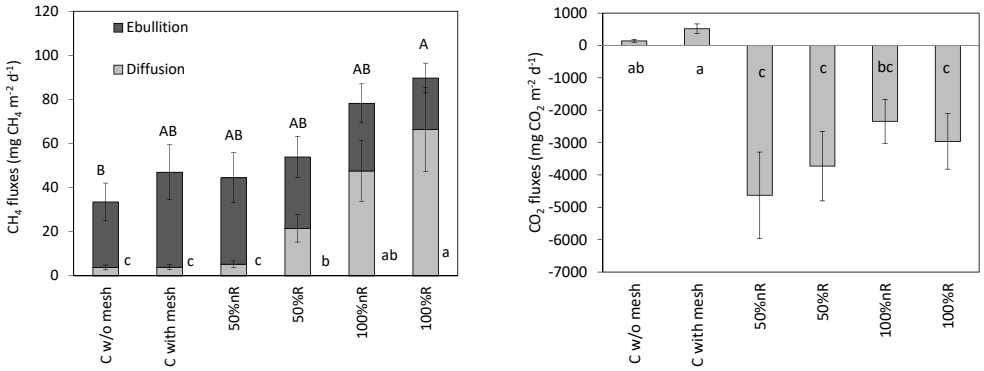


Figure 4.4. CH_4 (left) and CO_2 (right) fluxes (± SEM) for controls (C), low density (50%), and high density (100%) of water hyacinth with (R) or without rooting (nR) in the sediment. Different lower case letters and upper case letters indicate significant differences between treatments ($P < 0.05$) for diffusion and the total flux, respectively. No statistic difference was found for ebullition. Note different scales for the y-axis. Negative numbers refer to sequestration.

The effect of water hyacinth on oxygen and CH_4 emissions

Water hyacinth's cover lead to lower oxygen concentrations in the water column (Fig. 4.2). Low oxygen concentrations below other floating plant species have been reported in field and lab studies (Masifwa et al., 2001; Nahlik & Mitsch, 2006) and have been attributed to the suppression of O_2 diffusion across the air-water interface, decrease of primary production in the water column due to lower light availability and the high oxygen demand of decomposing plant material (Reddy & DeBusk, 1991).

Low oxygen concentrations may, however, result in increased CH_4 emissions (Bastviken et al., 2008) which may, at least partially, explain why the diffusive CH_4 emission was up to 17 times higher in aquaria with water hyacinth compared to the controls. Water hyacinth has previously been reported as a CH_4 enhancer (2 to 5 times more CH_4 emissions from water hyacinth mats compared to open waters) (Banik et al., 1993). Other studies, in contrast, showed 2.6 times higher CH_4 fluxes from open waters compared to from water hyacinth mats (Attermeyer et al., 2016). We postulate that this discrepancy may well be driven by different underlying mechanisms. For one, the densities might have differed, with higher densities leading to higher methanogenic rates. Additionally, along the roots of water hyacinth, CH_4 oxidation takes place due to the metanotrophic activity (Yoshida et al., 2014), and due to a radial oxygen loss provided by this plant (Kosten et al., 2016). Variation in root biomass and exudate loss, the composition and activity of microbial communities, and water and sediment composition can be expected to affect CH_4 oxidation rates and hence CH_4 emission rates. In our study, we found that rooting led to 1.3 and 4 times higher diffusive CH_4 emissions at high and low plant density, respectively, most possibly caused by the direct transportation of the CH_4 produced in the sediment to the atmosphere

through the aerenchyma of roots and leaves, thereby escaping CH_4 oxidation (Thomas et al., 1996; Bastviken et al., 2008).

Higher coverage led to higher diffusive CH_4 fluxes, presumably due to the production of (dissolved) organic matter substance and further lowering of O_2 concentrations. If the chimney effect indeed occurs, rooting in the sediment might avoid the formation of bubbles in the sediment, thereby decreasing ebullition and enhancing the proportion of CH_4 emitted by the plant tissue. We did, however, not find a significant effect of rooting on ebullition in our study.

The effect of water hyacinth on nutrient dynamics and carbon dioxide emissions

The percentage coverage by plants and their access to the sediment did not change nutrient uptake and allocation in a consistent way, although the high density rooting plants showed higher N: P ratios (Fig 4.3), due to higher N concentration (Supplementary Fig 4.2). Relatively high N concentrations under high-density conditions have been found in the field as well and have been related to N supply to the plant (Reddy et al., 1989). The general absence of a strong effect of root access to the sediment on plant nutrient contents suggests that the plants are capable of mobilizing nutrients from the sediment even without direct contact. This has also been demonstrated for the floating macrophyte *Stratiotes aloides*, for which the lowering of O_2 levels due to high coverage can promote P release from the sediment by weakening the bonds of Fe-P complexes (Harpenslager et al., 2016). The fact that water column nutrient concentrations tented to be higher in the treatments where the plants are rooted in the sediment (Table 4.2) suggests, however, that the plants preferably tap into the rich sediment nutrient pools directly. Porewater concentrations of N and P were 220 and 30 times higher than in the surface water (results not shown). *Egeria densa*, *Hydrilla verticillata*, and *Myriophyllum spicatum* have been reported to only take up P from the sediment (Barko & Smart, 1980).

More efficient nutrient uptake when rooted in the sediment could lead to higher growth rates and concomitant CO_2 sequestration. We did not find, however, higher CO_2 sequestration in our rooted treatments. We only found a clear difference between the densities, with on average 1.6 times lower CO_2 sequestration rates at high densities, which we attribute to the limited space for growth.

On average our plant treatments sequestrated -3.4 ± 2.2 g CO_2 m^{-2} day^{-1}, regardless of density and the position of the roots. This is notably higher than sequestration rates of other aquatic plants, such as *Typha domingensis* and *Eichhornia azurea*, showing sequestration rates around -0.09 g CO_2 m^{-2} day^{-1} (Gripp et al., 2013). The rates we measured are in the range between -3.4 and -5.4 g CO_2 m^{-2} day^{-1}, found for *E.c.* in field conditions (Attermeyer et al., 2016; Peixoto et al., 2016).

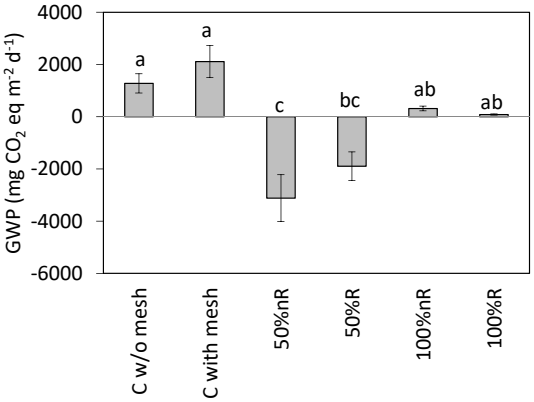


Figure 4.5. Global warming potential (GWP; mean ± SEM) for controls (C), low density (50%) and high density (100%) water hyacinth coverage with or without rooting in the sediment. Different lower case letters indicate significant differences between treatments ($P < 0.001$).

In aquaria without water hyacinth, CO_2 fluxes took place leading a 24h net emission of, on average, 0.3 g CO_2 m^{-2} day^{-1} (Fig 4.4). The net emissions from the non-vegetated controls contrast the net CO_2 sequestration in the plant treatments indicating that the plants offset the CO_2 emissions from open waters.

Effects of water hyacinth on the overall GHG balance

Under the experimental conditions of our study in the absence of water hyacinth, CH_4 emissions were modest and net CO_2 emissions took place, leading to an overall emission of GHG (Fig 4.5). At low density, however, water hyacinth was a net sink of GHGs, regardless of the position of the roots. At high density CO_2 sequestration only partially counterbalanced CH_4 emissions, thereby making the system become a small GHG source.

We here show that whether water hyacinth is a GHG sink or source depends on the balance of its effect on CH_4 emissions and CO_2 uptake rates. This balance, in turn, depends on density and whether or not they are rooting in the sediment with partially contradicting effects when it comes to CO_2 and CH_4. The plants tended to enhance CH_4 emissions especially at high density and when rooting in the sediment, whereas their CO_2 uptake rates were highest at low density where the growth was not space limited and nutrient availability per plant was higher.

Ebullition played an important role in the overall GHG balance, since it accounted, on average, for 58% of the total CH_4 emissions for all treatments and even reached 62% at low density. Underlining once more that ebullition is one of the most important forms of CH_4 contribution to the atmosphere (Coulthard et al., 2009).

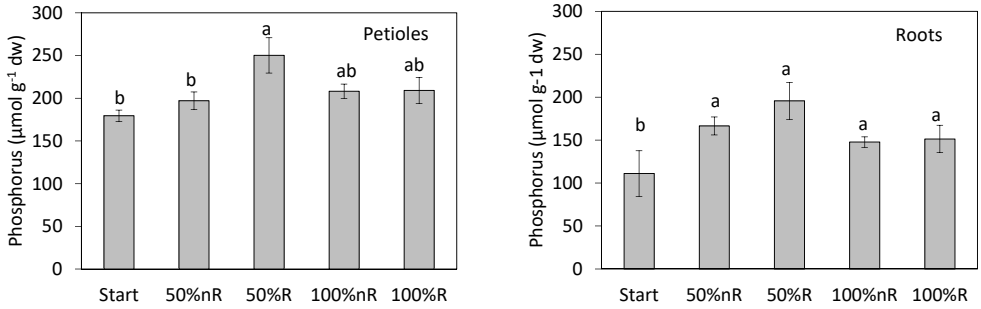
Our results highlight that the presence of water hyacinth mats can alter GHG emissions. CO_2

sequestration rates are enhanced and hence can trigger a regional effect offsetting the greenhouse gas emissions for open waters. Using water hyacinth for nutrient-rich wastewater purification under a relatively low density (like 50% coverage) by regular harvest will likely reduce the emission of CH_4 and increase the sequestration of CO_2, especially when roots are prevented from reaching the sediment. As a main conclusion, we here showed that access to the sediment, as related to water depth, and plant density are crucial factors influencing both nutrient dynamics and GHG emissions, which may explain the discrepancies reported in literature and should be taken into account when making regional GHG balances.

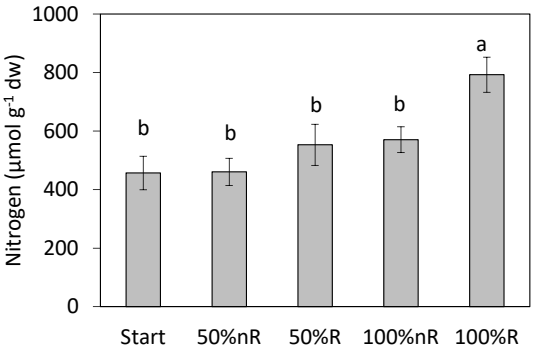
Acknowledgements

The authors would like to thank Walter Hendrickx, Yvette Evers for the Water hyacinth maintenance and Gerard van der Weerden for providing experimental space in the greenhouse facilities. We thank Paul van der Ven, Ankie De Vries-Brock, Germa Verheggen, Sebastian Krosse, and Roy Peters for their assistance with the chemical analyses. Han Wang was essential for the experiment implementation and data acquisition. Ernandes Sobreira was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES by the Science without Borders scholarship program Process BEX 13607/13-8, and the project was funded by Koninklijke Nederlandse Akademie van Wetenschappen (KNAW) and Fundação de Amparo a Pesquisa de Mato Grosso (FAPEMAT). Yingying Tang was funded by the China Scholarship Council (CSC; file number 201206140019). Sarian Kosten was supported by NWO-VENI grant 86312012.

Supplementary information



Supplementary Figure 4.1. Mean P concentrations (± SEM) in petioles (A) and roots (B) of water hyacinth for low density and high density with or without rooting in the sediment at the end and start of the experiment. Different lower case letters indicate significant differences between treatments including the start of the experiment ($P < 0.01$).



Supplementary Figure 4.2. Mean N concentrations (± SEM) in petioles of water hyacinth for low density and high density with or without rooting in the sediment at the end and start of the experiment. Different lower case letters indicate significant differences between treatments including the start of the experiment ($P < 0.001$).

Chapter 5

The roles of iron biogeochemistry and *Azolla* growth in the regulation of greenhouse gas, organic carbon and phosphorus emissions from rewetted peat soils

Yingying Tang, Monique M.L. van Kempen, Jan G.M. Roelofs, Sarian Kosten,

Alfons J.P. Smolders, Leon P.M. Lamers

Submitted

Abstract

Hypertrophic shallow wetlands are either dominated by algae or cyanobacteria, or covered by floating macrophytes such as water ferns (*Azolla* spp.), and may show high greenhouse gas (GHG) emissions. As the construction of new wetlands for water storage, flood protection and nature rehabilitation is often planned on former arable lands, excess phosphorus (P) stored in these soils will result in eutrophication of the overlying water after rewetting. Here, the active removal of P by the growth and harvest of fast growing *Azolla* spp. may be a viable scenario to avoid downstream eutrophication problems, recycle excess P, and decrease GHG emissions. Although it is well known that iron (Fe) is a key factor determining biogeochemical processes of wetlands, the exact factors and mechanisms regulating P, GHG and total organic carbon (TOC) emissions from *Azolla* dominated wetlands remain unclear. We therefore determined the mobilization rates of Fe and P into the water layer, their assimilation by *A. filiculoides*, and GHG and TOC emissions for two soils differing in P and organic matter contents, in a full factorial, controlled aquarium experiment. The soils that showed higher Fe mobilization, indicating high Fe reduction rates, also showed higher P mobilization and higher CO_2 and TOC production rates. P was partly taken up by *Azolla*, but P mobilization rates were unaffected by plant cover. In addition, high Fe-reduction soils showed 65% lower CH_4 emissions (diffusion plus ebullition) and 53% lower GHG emissions during the first 8 weeks, most probably due to the inhibitory effect of Fe on methanogenesis. Although it is well known that Fe reduction rates are often regulated by concentrations of oxalate-extractable Fe in soils, used as a proxy for the alternative electron acceptor Fe^{3+}, we found here that higher concentrations did not predict higher rates, but were determined by the specific composition of the available organic matter regulating electron donor availability. Our study not only shows that Fe related processes are key determinants of carbon and P biogeochemistry in rewetted agricultural peatlands, but also shows the importance of soil organic matter composition, i.e. the availability of easily decomposable organic matter. This information is not only important to understand the biogeochemistry of *Azolla*-dominated wetlands, but also for the application of *Azolla* sp. to recycle P from nutrient loaded agricultural soils after rewetting.

Introduction

The surface water of hypertrophic wetlands and lakes is generally dominated either by algae or cyanobacteria, or by floating macrophytes (Smolders et al., 2006; Kosten et al., 2012; Lamers et al., 2015). In addition, new wetlands are constructed for water purification, flood protection and ecological rehabilitation on former arable lands including peatlands that were heavily drained in the past (Smolders et al., 2008; Van Diggelen, 2015). As the application of fertilizer and manure has led to strong accumulation of phosphorus (P), the restoration of wetlands on former arable lands including drained peatlands could easily result in eutrophication of the overlaying water by the stimulation of P mobilization (Pant & Reddy, 2003; Geurts et al., 2010; Geurts et al., 2011). To deal with this, technologies are being developed that aim to remove P from agricultural lands (Dierberg et al., 2002; Sibrell et al., 2009). Compared with chemical P removal technologies applied in wastewater treatment, biological P removal technologies are more popular because of its cost effectiveness and environmental friendliness. P removal by plant uptake, especially by floating-leaved macrophytes, has widely been used for wastewater treatment in constructed wetlands (Ran et al., 2004; Polomski et al., 2009; Tang et al., 2016a). *Azolla filiculoides*, a floating fern that can potentially be used as a bio-fertilizer, is known to be very efficient in P removal because of its high P use efficiency and its ability to fix atmospheric N_2 by its symbiosis with the cyanobacteria *Anabaena azollae*, preventing nitrogen (N) limitation. As a result, it shows fast growth rates and is able to alleviate greenhouse gas (GHG) emissions by sequestrating carbon dioxide (CO_2) (Hill, 1977; Lumpkin & Plucknett, 1982; Pillai et al., 2005; Van Kempen, 2013).

As iron (Fe) is a key factor determining biogeochemical processes in wetlands (Loeb et al., 2007), it is important to gain insight into the role of differences in Fe reduction rates with regard to the mobilization of both P and dissolved organic carbon (DOC), and to the release of GHG in *Azolla* dominated wetlands. Under anaerobic conditions, Fe (III) is often the most important alternative electron acceptor for the decomposition of organic matter (Patrick Jr. & Khalid, 1974; Bostrom et al., 1982; Emsens et al., 2016). Due to a lower binding efficiency of Fe (II) for P compared to Fe (III), large amounts of both Fe and P can be released into the pore water in the process of Fe reduction (Loeb et al., 2007). Fe and P mobilized in anaerobic soils can diffuse into the surface water if the soil-water interface is anaerobic, preventing P re-sequestration by Fe (II) (Gunnars & Blomqvist, 1997; Smolders et al., 2006). Furthermore, as simple organic compounds from organic matter act as alternative electron donors for Fe (III) reduction, DOC production may be increased (Zak & Gelbrecht, 2007). Although it has been shown that the reduction of Fe (III) can induce both P and DOC mobilization (Patrick Jr. & Khalid, 1974; Loeb et al., 2007; Zak & Gelbrecht, 2007), the effect of the reduction of Fe (III) on GHG emissions (including both CH_4 and CO_2) is poorly understood.

Floating mats of *Azolla* sp. may prevent O_2 diffusion into the water by completely covering the water surface (Gratwicke & Marshall, 2001), thereby enhancing anaerobic conditions around

the soil-water interface and increasing P mobilization rates. Simultaneously, *Azolla* spp. can also sequestrate P from the surface water, thereby achieving P removal from P-rich wetlands including inundated former arable lands. Furthermore, *Azolla* sp. has a great potential to fix atmospheric carbon dioxide (CO_2) because of its fast growth resulting from the ample supply of P mobilized from the soil, and N fixed by *Anabaena azollae* (Speelman et al., 2009; Van Kempen et al., 2016). On the other hand, *Azolla* sp. may also enhance methane (CH_4) emissions by creating anaerobic conditions and providing high amounts of organic matter for methanogenesis (Furlanetto et al., 2012). At the same time, however, the roots of *Azolla* sp. can potentially oxidize produced CH_4, making the overall effect of *Azolla* sp. on CH_4 emissions not straightforward (Kosten et al., 2016).

In this study, we assessed the main biogeochemical processes regulating P and total organic carbon (TOC) mobilization, and GHG emissions in *Azolla*-dominated, rewetted peatlands. We used two different peat soils expected to show clear differences in Fe reduction rates, as estimated from data from an earlier study (Tang et al., 2016b). The objective of this study was to answer the following questions: (1) which factors regulate Fe reduction rates for rewetted peat soils, (2) do emissions of P, DOC and GHG differ between soils with different Fe reduction rates, and (3) how does *A. filiculoides* affect P and TOC mobilization, and GHG emissions? The outcome is not only important to understand biogeochemical processes in *Azolla*-dominated wetlands, but also to assess the potential use of *Azolla* spp. to recycle P from rewetted agricultural land.

Materials and methods

Experimental set-up

In total, 32 peat monoliths (25 × 12.5 × 10 cm; l × w × h) were collected in February 2015 in Zuidplas (51°59′N, 4°39′E) and in Burkmeer (52°25′N, 4°59′E). From data from a previous study (Tang et al., 2016b), we were able to estimate clearly different Fe mobilization rates of 6.2 and 54.6 μmol m^{-2} d^{-1} for Zuidplas and Burkmeer, respectively. Soil characteristics are displayed in Table 5.1, expressed per unit volume to enable comparison between soils. After collection, the soils were transported to the greenhouse facility at Radboud University (Nijmegen, The Netherlands) and transferred into 32 glass aquaria (25 × 12.5 × 30 cm; l × w × h; n = 16 for each location). Next, 4.7 L of a standardized water containing 1000 μmol L^{-1} $NaHCO_3$, 1000 μmol L^{-1} $CaCl_2.2H_2O$, 250 μmol L^{-1} KCl, 500 μmol L^{-1} $MgSO_4.7H_2O$, 10 μmol L^{-1} Fe-EDTA, 10 μmol L^{-1} NaCl, 1 μmol L^{-1} $CuSO_4.5H_2O$, 20 μmol L^{-1} $MnCl_2.4H_2O$, 10 μmol L^{-1} $ZnSO_4.7H_2O$, 3 μmol L^{-1} $Na_2MoO_4.2H_2O$, 20 μmol L^{-1} H_3BO_3, and 4 μmol L^{-1} $CoCl_2.6H_2O$ (De Lyon & Roelofs, 1986) was carefully added to all aquaria. *A. filiculoides* (18.12 ± 0.00 g FW; 1.09 ± 0.00 g DW; water fern, floating macrophyte) was added to half of the aquaria (n = 8) for each location. The aquaria were kept at 20°C by placing them inside a water bath (Neslab Merlin M 100; Thermo Scientific, Newington, NH, USA) at a light

Table 5.1. Soil characteristics of *Burkmeer* and *Zuidplas* soils used in the experiment (± SEM; n = 8). Significant differences between soil types are indicated using asterisks, with * representing $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and NS not significant.

Soil	Bulk density (kg DW.L^{-1} FW)	Organic matter (kg DW.L^{-1} FW)	Oxalate extractable Fe (mmol L^{-1} FW)	Oxalate extractable P (mmol L^{-1} FW)	Total-Fe (mmol L^{-1} FW)	Total-P (mmol L^{-1} FW)
Burkmeer	0.28 (0.01)	0.19 (0.01)	88.38 (2.76)	11.05 (0.50)	83.49 (6.05)	19.54 (1.42)
Zuidplas	0.50 (0.01)	0.17 (0.00)	94.38 (5.59)	35.59 (1.25)	112.37 (7.68)	43.83 (2.77)
Significance	***	*	NS	***	**	***

intensity of a minimum of 230 µmol m^{-2} s^{-1} PAR (16h light/8h dark; Quantum sensor, Skye Instruments LTD, Wales, England) provided by Philips Green Power 400V/1000 WE lamps in a New E-Papillon 1000W armature in case of cloudy conditions. Throughout the article, soils will be called *Zuidplas* (expected to show low Fe reduction rates) and *Burkmeer* (high Fe mobilization rates).

Plant measurements

During the experimental period, total fresh weight of *A. filiculoides* in each aquarium was recorded once every two weeks for the first two weeks and once every week for the last six weeks. At these times we harvested part of the biomass to avoid space limitation, leaving 18 g (the starting biomass of the experiment) of fresh *A. filiculoides* in the aquarium. The dry weight of harvested *A. filiculoides* was determined after drying for 48 h at 60°C, and subsequently the material was ground and homogenized for chemical analyses.

Chemical analyses

Surface water samples were collected every week between March and April 2015, after which fresh surface water solution was added to compensate for the sampling volume. Pore water samples were collected every week at 0–2 cm below soil surface, using ceramic soil moisture samplers (SMS rhizons, Eijkelkamp, Giesbeek, Netherlands). After 1 week, oxygen (O_2) concentrations in the surface water were measured every week at 7 cm below the water surface in all aquaria with an optical oxygen probe (Hach LDO, Hach, Loveland, CO, U.S.A.). Total organic carbon (TOC) in unfiltered surface water samples and dissolved organic carbon (DOC) in filtered pore water samples were measured by a TOC-L CPH/CPN analyzer (Shimadzu, Kyoto, Japan). Surface water samples were filtered through 1.2 μm pore size glass microfiber filters (GF/C, Whatman International Ltd., England) using a vacuum pump (Vacuubrand, Wertheim, Germany). Concentrations of dissolved total Fe and P in filtered surface water and in pore water were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES; IRIS Intrepid II, Thermo Fisher Scientific, Franklin, MA, U.S.A.).

At the end of the experiment, soil samples were collected, volume weighed and dried for 48 h at 60°C to determine bulk density, after which they were ground with a mortar and pestle for further analyses. Crude ash was determined by the Weende method (Helrich, 1990), whereas neutral detergent fiber (NDF), acid detergent fiber (ADF), and detergent lignin (ADL) were determined according to the method of Van Soest et al. (1991). Cell soluble (dry weight-NDF-crude ash), lignin (ADL), cellulose (+ cutine) (ADF-ADL) and hemicellulose (NDF−ADF) were calculated. Dry soil samples were heated (4 h, 550°C) and subsequently re-weighed to determine organic matter content. Furthermore, 200 mg of dry soil was digested with 4 mL 65% HNO_3 and 1 mL 30% H_2O_2 using a microwave oven (MLS-1200 Mega, Milestone Inc., Sorisole, Italy), after which digestates were analyzed by ICP-OES (see above) to determine total sediment Fe and P concentrations. The concentrations of amorphous Fe and Fe-bound and aluminum- (Al-) bound P were determined by oxalate extraction according to Houba et al. (1989). 200 mg of dry plant material was digested and subsequently analyzed as described above to determine total-P and Fe concentrations in plants. Cumulative biomass production and P content of *A. filiculoides* were used to calculate *A. filiculoides* sequestration rates of P.

GHG flux measurements

Diffusive fluxes of CO_2 and CH_4 were measured 5 times under light and dark conditions using a lid on top of the aquarium, connected to a Greenhouse Gas Analyzer (Picarro G2508, Picarro Inc., Santa Clara, CA, USA) to establish a closed loop. The lid/aquarium connection was airtight by sealing with Terostat paste (Terostat IX, Teroson GmbH, Heidelberg, Germany). For dark measurements, aquaria were covered with opaque fabric to mimic dark conditions. To ensure dark adaption of *A. filiculoides*, measurements were conducted 40 minutes after creating dark

conditions. Measurements were conducted until a clear linear increase in CO_2 and CH_4 occurred (typically for 5 minutes). The slope of the relationship between gas concentration and time was used to calculate the gas flux as in Almeida et al. (2016). When the linear increase was interrupted by a sudden increase in gas concentration – due to bubbling – the lid was removed to lower the gas concentrations in the gas space and the diffusive flux measurement was repeated.

Total CH_4 fluxes (diffusion plus ebullition) were measured 6 times during a period of 24 h. During the 24 h period the glass lid with a rubber septum covered the top of each aquarium and the lid/aquarium connection was sealed with Terostat paste (see above). The headspace gas of each aquarium was sampled (in triplicate) at the start and end of the 24 h incubation, and subsequently analyzed by a gas chromatograph (HP 5890 equipped with a Porapak Q column (80/100 mesh), a flame ionization detector (Hewlett Packard, USA), and an oven temperature of 120°C) to determine the increase in CH_4 concentration. All gas fluxes were corrected for headspace volumes. GWPs were estimated by measured fluxes, and expressed as CO_2-eq m^{-2} d^{-1}, using a GWP for CH_4 34 times that of CO_2 on a mass basis over a 100 year period (Stocker et al., 2013).

Statistical analyses

Data were tested for normal distribution of residuals and homogeneity of variance using the Shapiro-Wilk test and Bartlett's test, respectively. Non-normal or heteroscedastic data were log transformed to meet the assumptions of ANOVA. Linear mixed models (R package nlme) were used to test the main effects and interactions of treatments over time on surface water O_2 and TOC concentrations, pore water DOC concentrations, DTP and Fe concentrations in both surface and pore water, Fe and P concentrations in *A. filiculoides*, total CH_4 fluxes, diffusive CO_2 fluxes (for this response variable not including time as a main effect in this model), and GWPs, with aquarium number as a random effect. Differences in organic matter fractions and other soil characteristics between soil types were also tested using linear mixed models, with aquarium number as a random effect. Effects were considered significant if $P < 0.05$. Differences between treatments were tested by Tukey tests at the 0.05 level of significance using R package multcomp. All statistical analyses were performed using the software program R (version 3.2.1; R development Core Team, 2015), and graphs were plotted using R package ggplot2.

Results

Surface water and pore water characteristics

Time, plant presence, or soil type did not show effects on surface water O_2 concentrations ($P > 0.05$; data not presented); the average surface water O_2 concentration was 0.90 ± 0.20 mg L^{-1}

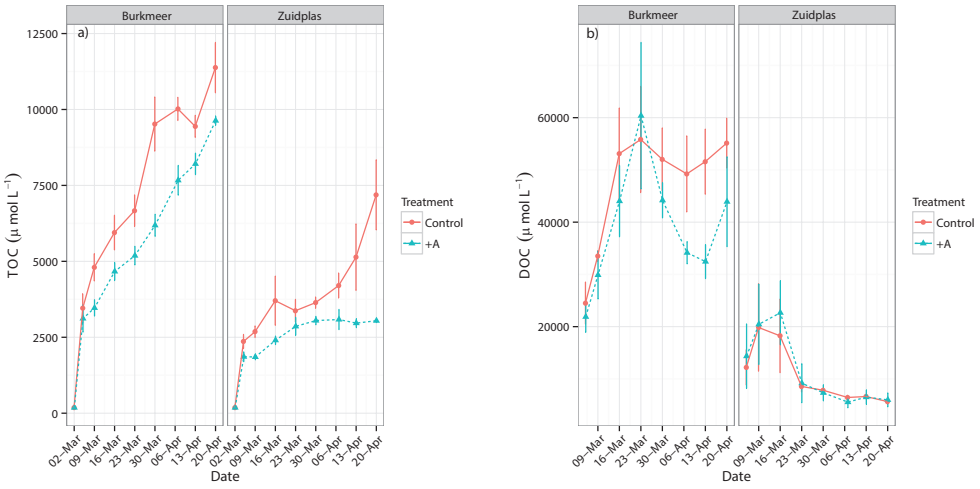


Figure 5.1. Average surface water TOC (a) and pore water DOC (b) concentrations in aquaria with *A. filiculoides* (+A) or without *A. filiculoides* (Control) for *Burkmeer* or *Zuidplas* soils after flooding. Vertical lines represent SEMs. Note different scales for y-axes.

during the experiment for all treatments. Over time, surface water TOC concentrations increased strongly (Fig. 5.1a; $X_2 = 86.68$, $P < 0.001$). *Burkmeer* soils showed 2 times higher TOC concentrations in the surface water and 3.8 times higher DOC concentrations in the pore water than *Zuidplas* soils (Fig. 5.1; $X_2 = 26.41$, $P < 0.001$ and $X_2 = 8.04$, $P < 0.01$ for TOC and DOC respectively). Although surface water TOC concentrations did not significantly differ between treatments with and without *A. filiculoides* ($P = 0.10$), they tended to be slightly lower in the treatments including *A. filiculoides*.

Burkmeer soils showed 3.7 times higher Fe concentrations in the surface water and 8.8 times higher Fe concentrations in the pore water than *Zuidplas* soils after rewetting (Fig. 5.2; $X_2 = 25.64$, $P < 0.001$ and $X_2 = 34.35$, $P < 0.001$ for surface water and pore water respectively). For *Burkmeer*, Fe concentrations in the surface water were significantly lower in aquaria including *A. filiculoides* ($X_2 = 59.14$, $P < 0.001$). Furthermore, in aquaria with *A. filiculoides* there was a lower DTP concentration in the surface water for both soil types (Fig. 5.3a; $X_2 = 25.05$, $P < 0.001$), whereas DTP concentrations in the pore water did not differ with and without plants (Fig. 5.3b; $P > 0.05$). Although initial DTP mobilization rates for surface water and pore water were similar for both soils ($P = 0.38$ and $P = 0.71$, respectively), surface water and pore water DTP concentrations were higher for *Burkmeer* at the end of the experiment, because they remained high while those for *Zuidplas* strongly decreased ($X_2 = 8.79$, $P < 0.01$ and $X_2 = 10.71$, $P < 0.01$ for surface water and pore water respectively).

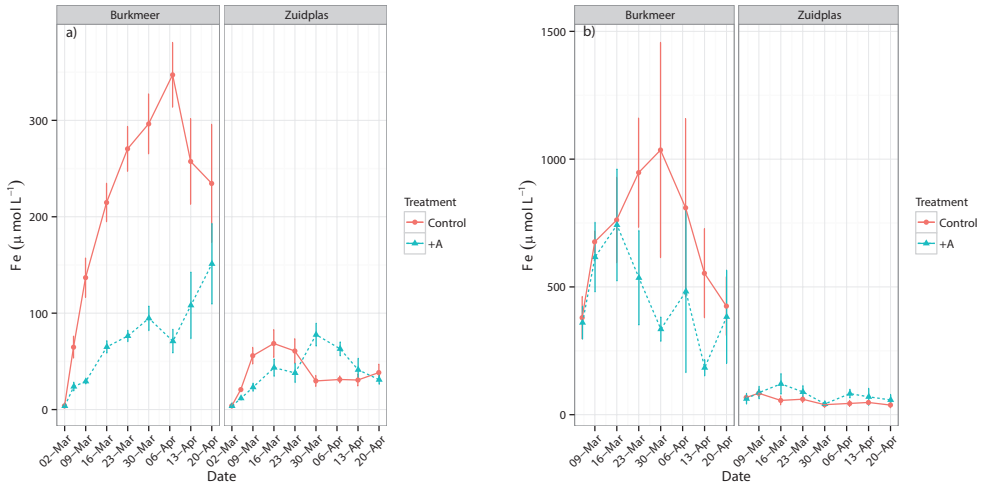


Figure 5.2. Average surface water Fe (a) and pore water Fe (b) concentrations in aquaria with *A. filiculoides* (+A) or without *A. filiculoides* (Control) for both soils. Vertical lines represent SEMs. Note different scales for y-axes.

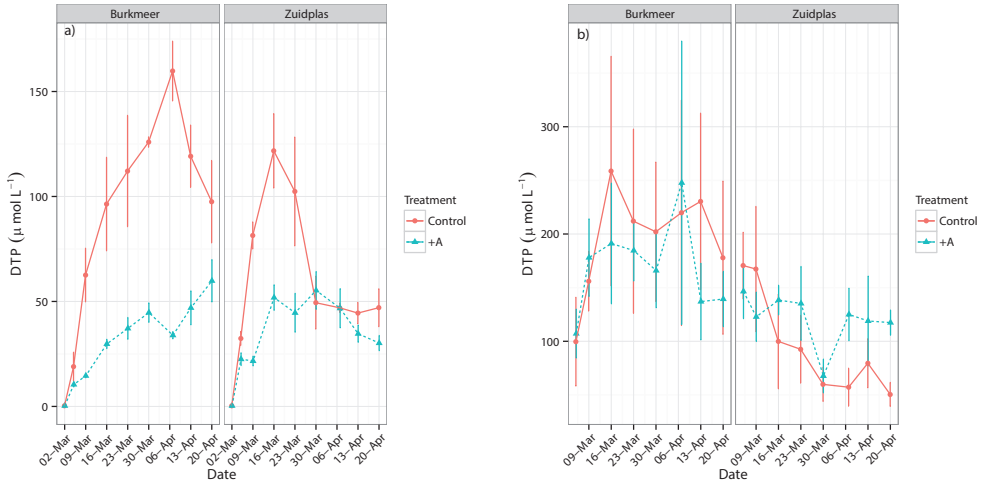


Figure 5.3. Average surface water DTP (a) and pore water DTP (b) concentrations in aquaria with *A. filiculoides* (+A) or without *A. filiculoides* (Control) for both soils. Vertical lines represent SEMs. Note different scales for y-axes.

Azolla growth and nutrient assimilation

There were no significant differences in cumulative dry weight of *A. filiculoides* between the two soils ($P = 0.91$; data not shown). On average, growth rates were 3.4 ± 0.1 g DW m^{-2} d^{-1}. However, *A. filiculoides* grown on *Burkmeer* soil showing higher Fe mobilization rates, had 2.5 times higher Fe concentrations than for *Zuidplas* ($X_2 = 61.89$, $P < 0.001$; Fig. 5.4a). *A.*

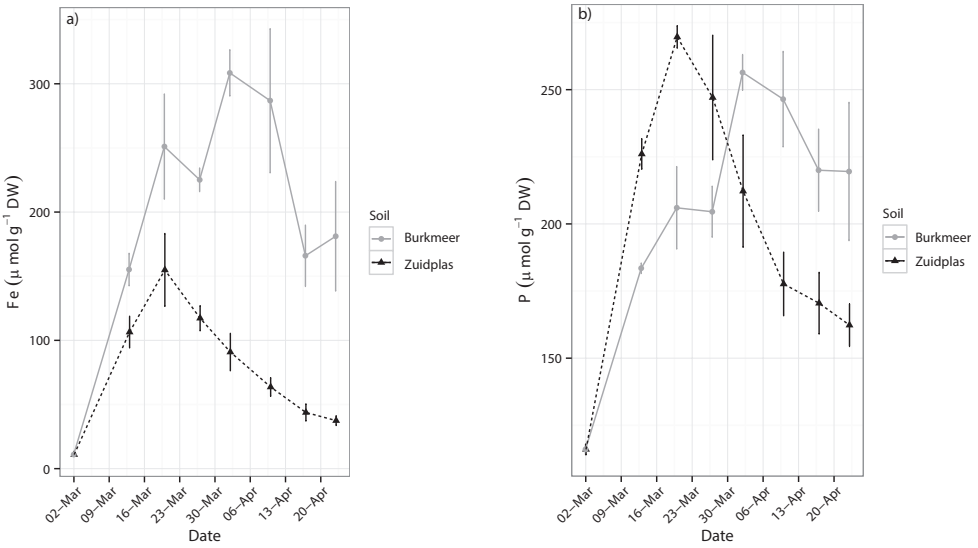


Figure 5.4. Average Fe (a) and P (b) concentrations in *A. filiculoides* grown for both soils. Vertical lines represent SEMs. Note different scales for y-axes.

filiculoides showed maximum Fe and P concentrations (308.5 ± 18.0 μmol Fe g^{-1} DW and 256.4 ± 6.6 μmol P g^{-1} DW) at day 31 for *Burkmeer* after which concentrations started to decrease. For *Zuidplas*, plants showed maximum Fe and P concentrations (154.9 ± 28.3 μmol Fe g^{-1} DW and 269.6 ± 4.2 μmol P g^{-1} DW) at day 18 (Fig. 5.4). Average P sequestration rates by *A. filiculoides* during the experiment were 0.77 ± 0.03 mmol P m^{-2} d^{-1} and did not significantly differ between both soils.

GHG emissions

Although *Burkmeer* soils (with higher Fe mobilization rates) only had slightly higher organic matter contents (Table 5.1; $X_2 = 6.55$, $P < 0.05$; Fig 5.5a), they showed 3.1 times higher diffusive CO_2 emissions to the atmosphere than *Zuidplas*. *A. filiculoides* significantly reduced diffusive CO_2 emissions to the atmosphere compared to the absence of *A. filiculoides* ($X_2 = 3.95$, $P < 0.05$), due to photosynthesis (−742 ± 204 vs 763 ± 170 mg C m^{-2} d^{-1} for daytime measurement, regardless of soil type). Aquaria with *A. filiculoides* growing above *Burkmeer* soil (highest Fe reduction rates), always functioned as a CO_2 source, whereas in aquaria with *Zuidplas* soils CO_2 was sequestrated during the last three sampling times. At the same time, *Burkmeer* soils had 65% lower total CH_4 emissions (diffusion plus ebullition; calculated by area under the curve) ($X_2 = 9.32$, $P < 0.01$; Fig. 5.5b). By the end of the experiment, however, CH_4 emissions had become similar for both soils. As a result of decreasing CH_4 emission, GWP of *Zuidplas* decreased during the experiment ($X_2 = 29.21$, $P < 0.001$; Fig 5.6). Although GWP due to GHG emitted

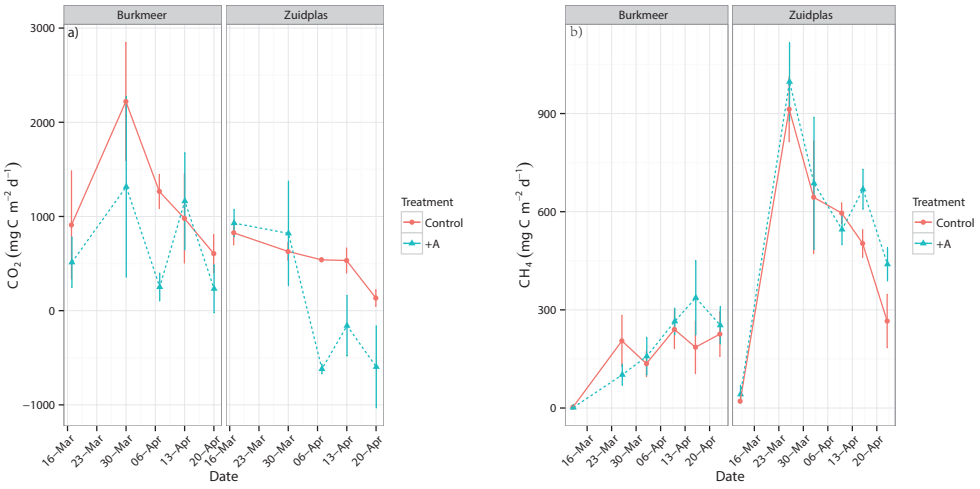


Figure 5.5. Average diffusive CO_2 fluxes (a) and total CH_4 emissions (diffusion plus ebullition) (b) basing on weighted average of day and night time fluxes in aquaria with *A. filiculoides* (+A) or without *A. filiculoides* (Control) for both soils. Vertical lines represent SEMs. Note different scales for y-axes. Positive values represent CO_2 release; negative values represent CO_2 fixation.

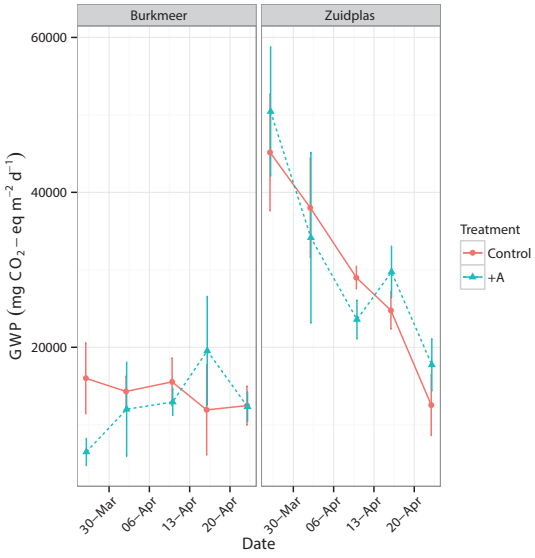


Figure 5.6. Average global warming potential (GWP) in aquaria with *A. filiculoides* (+A) or without *A. filiculoides* (Control) for both soils. Vertical lines represent SEMs.

from *Burkmeer* was initially lower than that *Zuidplas* ($X_2 = 18.63$, $P < 0.001$), the GWPs for both soils became similar after 8 weeks, regardless of the presence of *A. filiculoides*.

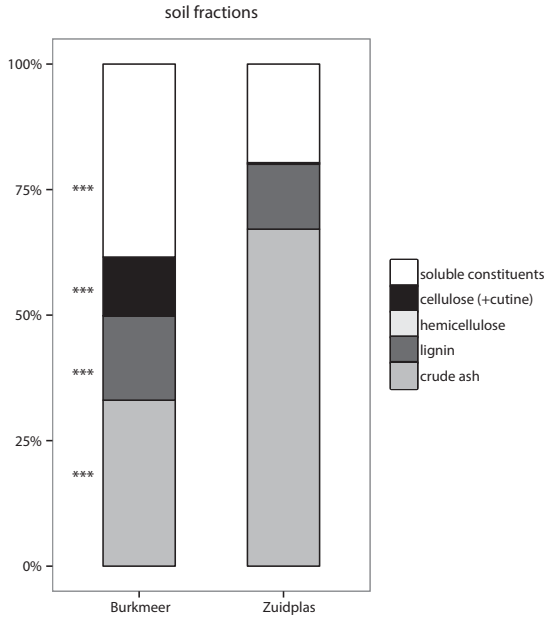


Figure 5.7. Soil organic matter fractions for *Burkmeer* and *Zuidplas* soils. Significant differences between the two soils are indicated with asterisks, with *** representing $P < 0.001$ (n = 8).

Soil characteristics and composition

Unexpectedly, the *Burkmeer* soil showing higher Fe and P mobilization rates had similar oxalate-extractable Fe concentrations (indicative of the availability of Fe as an electron acceptor), and only slightly higher organic matter content (Table 5.1). Moreover, *Burkmeer* showed lower oxalate-extractable P, and lower total Fe and P concentrations (Table 5.1). We therefore analyzed the organic matter composition for both soils. This showed that *Burkmeer* soil contained higher weight fractions of soluble constituents (cell contents, including soluble carbohydrates, protein, lipids, minerals and vitamins), cellulose (+ cutine), and lignin than *Zuidplas* soil, whereas it contained lower weight fractions of crude ash (Figure 5.7). Both soil types contained only small amounts of hemicellulose (less than 0.2%) (Figure 5.7).

Discussion

Eutrophic wetlands and rewetted agricultural soils often show high P mobilization and GHG emission. Our study showed that P mobilization rates, GHG emission and TOC production were strongly linked to Fe reduction rates in rewetted peat soils. Differences in Fe reduction rates could most probably be explained by differences in electron donor availability, rather than by

differences in Fe and P availability. Cover of the water layer by *Azolla* led to sequestration of Fe, P, and C, but did not affect mobilization rates.

Regulation of Fe reduction rates

Earlier studies have shown a strong correlation between Fe and P mobilization rates, and concentrations of amorphous Fe in the soil (Zak & Gelbrecht, 2007; Emsens et al., 2016). In our study, however, we found significant differences in Fe reduction rates without differences in oxalate-extractable Fe contents between both soils (Table 5.1), showing that Fe reduction rates are not only regulated by the availability of amorphous Fe as an electron acceptor. For both tested soils, total organic matter contents per liter soil were almost similar (Table 5.1). However, analysis of the organic matter composition showed that the soil with higher Fe reduction rates had higher weight fractions of soluble constituents and cellulose (Figure 5.7). These constituents are regarded as relatively easily decomposable organic matter, releasing simple organic compounds such as acetate and lactate that serve as electron donors for microbial redox reactions (Swift et al., 1979; Richardson, 1985; Lamers et al., 2001). This shows that next to the availability of Fe as an electron acceptor, the composition of soil organic matter may also regulate Fe reduction rates.

Fe-mediated P mobilization and GHG emission

In the *Burkmeer* soil Fe reduction played an important role due to the availability of easily degradable organic matter, which is also shown by high DOC concentrations in the pore water. Due to the higher activity of the Fe reducing community, methanogenesis and CH_4 emission (diffusion plus ebullition) were suppressed (Van Bodegom et al., 2004; Zhang et al., 2009). In the *Zuidplas* soil, Fe reducing communities were much less active, probably because of limited availability of easily degradable organic matter (Fig. 5.7, and also shown by low and decreasing porewater DOC levels in Fig. 5.1). As a result, methanogenic bacteria became active more rapidly in this soil, leading to a higher but transient peak in CH_4 emission.

C dynamics and GHG emissions from *Azolla*-dominated wetlands

Although *A. filiculoides* was taking up CO_2 by photosynthesis, 0.70 ± 0.24 g CO_2-C m^{-2} d^{-1} was still emitted into the atmosphere on a daily basis from aquaria with *A. filiculoides* growing on the soil that showed highest Fe reduction rates (*Burkmeer*). Although this soil contained only a slightly higher level of organic matter, it had much higher concentrations of cellulose and soluble constituents (Table 5.1, Figure 5.7). Therefore, higher decomposition rates of labile organic matter, providing simple organic compounds acting as electron donors, likely produced more CO_2 than could be taken up by the plants (Jacinthe et al., 2002; Ludwig et al., 2011). In combination with the presence of reactive Fe, anaerobic oxidation rates were enhanced (Lovley &

Phillips, 1988). Therefore, *Azolla*-dominated wetlands can still function as a CO_2 source due to higher decomposition rates. For *A. filiculoides* grown on *Zuidmeer* soils, showing much lower Fe reduction rates and decomposition rates, CO_2 sequestration by photosynthesis of *A. filiculoides* counterbalanced CO_2 emissions during the second half of the experiment (Jäger et al., 2011), changing the system to a C sink. The total CH_4 emissions we measured for aquaria with *A. filiculoides* regardless of soil type, ranging from 0.03 to 1.28 g C m^{-2} d^{-1}, are comparable to, or even higher than, values of 0.03 to 0.06 g C m^{-2} d^{-1} reported for free floating macrophyte mats (including *Azolla pinnata*) in man-made wetlands (Singh et al., 2000). For *Zuidplas* soil, total CH_4 emissions started to decrease after day 24, which may be caused by limited availability of organic compounds such as acetate for methanogenic communities (Van Der Gon & Neue, 1995; Medvedeff et al., 2014). This shows that *Azolla*-dominated wetlands may act as either C sources or sinks, depending on their decomposition rates. GWP were, however, positive for both soils.

Implications for management

Higher Fe reduction rates led to higher Fe and P mobilization rates in pore water and surface water, and both elements were partly consumed by *Azolla*. Although CO_2 emissions were higher and CH_4 emissions were lower for soils showing strong Fe reduction, GHG emissions became similar after 2 months for both soils differing in Fe reduction rates. *A. filiculoides* cover lowered CO_2 emission, but did not affect CH_4 emission.

P is a non-renewable resource that is becoming scarce at a global scale (Petzet et al., 2012; Hao et al., 2013), and it is estimated that current rock phosphate reserves will be exhausted in 50–100 years (Smil, 2000; Cordell et al., 2009). This means that P recycling becomes more and more important. In this study, we showed P sequestration rates by *A. filiculoides* of 0.8 mmol P m^{-2} d^{-1} (25 mg P m^{-2} d^{-1}), showing that *Azolla* spp., that can be used as green fertilizer, may be useful for P recovery from P loaded soils after rewetting.

Conclusions

In conclusion, we show that Fe reduction rates on rewetted peat soils are not only determined by contents of oxalate-extractable Fe in soil, but also by the composition of soil organic matter. Fe reduction rates are strongly linked to the regulation of GHG, DOC and P emission. Although the cover of the water layer by *Azolla* leads to C, P and Fe sequestration, rewetted peat soils may still act as C and GHG sources due to their high anaerobic decomposition rates.

Acknowledgements

The authors would like to thank Daniel Hernádez Jack and Han Wang for their practical assistance, Ralf Aben for his help with the GHG flux calculations and Ankie De Vries-Brock, Germa Verheggen, Paul Van der Ven, Roy Peters and Sebastian Krosse for their assistance with chemical analyses. YT was funded by the China Scholarship Council (CSC; file number 201206140019). SK was funded by NWO-VENI grant 86312012.

Chapter 6

Synthesis: an applied perspective on wetland functioning

Yingying Tang

Using macrophyte-dominated wetlands for nutrient removal

Due to their high productivity and ability to sequester atmospheric CO_2, and their effective nutrient assimilation by plant uptake, macrophyte-dominated wetlands are globally being used for nutrient removal from polluted water. In most cases, helophytes (emergent aquatic macrophytes) are used in constructed wetlands. While helophytes mainly absorb nutrients from the underwater soil, floating and submerged aquatic macrophytes, such as *Azolla* spp. or *Chara* spp., can also effectively absorb nutrients from the water layer (Knight et al., 2003; Van Kempen et al., 2012), and may therefore be more effective. As experimental knowledge about the use of floating and submerged macrophytes for nutrient removal is sparse, part of the research in this thesis focused on their effectiveness in relation to nutrient availability in surface water and soil. By regularly harvesting these plants, nutrients originating from both inflowing water and soil can be withdrawn from the wetland system.

Effectiveness in relation to external nutrient loading

Nutrient removal efficiencies of macrophytes strongly depend on external nutrient loadings (Gottschall et al., 2007; Brisson & Chazarenc, 2009). In Chapter 3, we have shown that in macrophyte-dominated constructed wetlands, submerged or floating macrophytes were able to remove most of the added nutrients at low phosphorus (P) and nitrogen (N) loading rates. At high nutrient loadings, however, these macrophytes could only remove up to 45% (P) and 65% (N) of the external loads. This shows that wastewater with high nutrient contents can only be cleaned efficiently if very large areas are available. In most cases, however, aquatic macrophytes can only be used for wastewater polishing (final step of purification), but not as a stand-alone treatment for nutrient removal from wastewater. In order to effectively remove nutrients from wastewater with high nutrient loads, one should consider combining artificial wetland systems with various physicochemical and biological pre-treatment measures. Furthermore, we showed that only fast growing aquatic macrophytes such as *Azolla filiculoides* or *Myriophyllum spicatum* can efficiently contribute to nutrient removal, which indicates that the selection of macrophyte species deserves more attention (Chapter 3; Brisson and Chazarenc (2009)).

Another important finding is that wetland soils appear to be equally important in retaining P in macrophyte-dominated wetlands, but may also release substantial amounts of nutrients. Wetland soils can therefore serve as nutrient sources or sinks, depending on external P loadings (Reddy & D'Angelo, 1994). At low external P loading, wetland soils are sources of P, whereas they become P sinks at higher P loadings (Chapter 3). Although soils release P rather than retain P at low loading rates, 100% of all P, including both released P and external P loadings, can still be taken up by the vegetation. This means that P mobilization from sediments will not lead to a deterioration of water quality (Chapter 3). Furthermore, P retention by wetland soils also depends on physicochemical

characteristics of wetland soil (Reddy & DeLaune, 2008). In Chapter 3, we have shown that clay soils can immobilize P much better and become saturated less quickly than more organic soils. However, the nutrients immobilized by clay soils cannot directly be harvested. If floating macrophytes such as *Lemna* sp. and/or *Azolla* sp. are used, the accumulation of especially P in the soils can be prevented if P loads are low, as shown in Chapter 3.

Effectiveness in relation to internal nutrient loading

Aquatic macrophytes can indirectly or directly take up P from the soils (Chapter 3; Carignan and Kalff (1980); Harpenslager et al. (2016)), thereby lowering the risk of internal eutrophication and realizing P mining in the process of wetland creation and restoration. In macrophyte-dominated wetlands, floating macrophytes can strongly contribute to Fe-mediated P mobilization from soils into the overlying water, due to anaerobic conditions created by its coverage (Chapter 4 and 5; Reddy and DeBusk (1991); Caraco et al. (2006)). In chapter 5, we showed that oxygen (O_2) concentrations in the surface water became as low as 0.64 mg/L in aquaria with *A. filiculoides* for two different organic soil types, and that large amounts of P were simultaneously mobilized from these soils into the water. P mobilization was strongly coupled to microbial Fe reduction, and rates appeared to be regulated by organic matter quality. Furthermore, rooted submerged macrophytes, such as *M. spicatum*, efficiently take up nutrients from the soils directly (Best & Mantai, 1978; Barko & Smart, 1980; Carignan & Kalff, 1980), which, as we showed in Chapter 3, is the other way to extract P from the soils. Although the pathways for internal P mining are different, P sequestration rates by *A. filiculoides* (indirect uptake after mobilization of P to the water layer) and *M. spicatum* (direct uptake from sediment pore water) were still comparable when soils served as sources of P (Chapter 3). Therefore both fast growing rooted submerged macrophytes as well as floating macrophytes are good candidates to efficiently extract P from eutrophic soils in constructed wetlands and after rewetting of agricultural lands.

Soil characteristics play an important role in modulating P mobilization to the overlying water layer (Smolders et al., 2006; Loeb et al., 2007). Under more or less aerobic surface water conditions, P mobilization to the overlying water layer can be determined by P in the soil water (water-extractable P) (Chapter 2), whereas under anaerobic surface water conditions, the reduction of Fe (III) may regulate P mobilization (Zak & Gelbrecht, 2007; Loeb et al., 2008). In addition to Fe, labile soil organic matter is another contributor to P mobilization, as we showed in Chapter 2 and 5. Simple organic compounds derived from this fraction serve as an important electron donor for the reduction of Fe (III) (Lamers et al., 2002a; Zak & Gelbrecht, 2007). Furthermore, being a microbially mediated process, P mobilization also depends on temperature (Holdren & Armstrong, 1980; Boers & Van Hese, 1988; Liikanen et al., 2002). In Chapter 2 we found an average Q_{10} (temperature coefficient) of 2.8 for P mobilization rates of rewetted soils, independent of the soil types.

As indicated, we showed (Chapter 2) that the release after rewetting could easily be predicted by the water extractable P concentration of the soil. As this P fraction usually decreases in depth in agricultural soils as a result of the fertilization legacy, topsoil removal can be an effective measure to create P limitation and thereby prevent P mobilization upon inundation (Lamers et al., 2006; Smolders et al., 2008; Emsens et al., 2015). Alternatively P rich soils can be inundated and used for the growth of *A. filiculoides* (Chapter 5). This might provide an interesting cost-effective alternative for top soil removal. For decision support with respect to optimal land management, our method provides an easy, fast and cost-effective way to estimate internal P mobilization rates after rewetting.

Using macrophyte-dominated wetlands for C sequestration

In addition to nutrient removal, both natural and constructed wetlands can also sequester and store C from the atmosphere (Chapter 3; Whiting and Chanton (2001); Mitsch et al. (2013)). Wetlands may therefore serve as a C sink as CO_2 assimilation by macrophytes may offset decomposition, leading to net C accumulation in the soil C pool (Brix et al., 2001; Frolking et al., 2006). However, wetlands can still be a greenhouse gas (GHG) source as CH_4 is emitted to the atmosphere during anaerobic organic matter decomposition, and this gas has a much higher global warming potential (Poffenbarger et al., 2011; Bernal & Mitsch, 2012). Therefore, the overall GHG balance between net CO_2 fluxes and CH_4 emission determines whether a wetland system is a net GHG sink or a net GHG source. Especially the creation of new wetlands on drained peat soils can strongly reduce net GHG emissions as the aerobic oxidation of organic matter is stopped (Ström et al., 2005). In this thesis, we mainly focused on C fluxes in floating vegetation.

CO_2 fluxes in floating vegetation

Although high production rates lead to high C sequestration rates, high plant density may also decrease plant growth due to space limitation and self-shading, thereby lowering photosynthetic CO_2 assimilation (Chapter 4; DeBusk et al. (1981); Driever et al. (2005)). In Chapter 4, we found this for *Eichhornia crassipes* with different densities, with 1.6 times lower CO_2 assimilation rates at high densities. Both *E. crassipes* and *A. filiculoides* have a huge potential to sequestrate atmospheric CO_2. Nevertheless, we showed that *E. crassipes* can indeed be a net sink of CO_2 (Chapter 4), while *A. filiculoides* growing on Fe rich organic soil acts as a net source of CO_2 (Chapter 5). This can be explained by the high decomposition rates induced by a high availability of labile soil organic matter in Chapter 5 (Jacinthe et al., 2002; Jäger et al., 2011). This shows that both plant density and the availability of labile organic matter are important factors determining net CO_2 fluxes, at least in the short term.

CH_4 fluxes in floating vegetation

In deep waters, 30–99% of the CH_4 produced in the anoxic environment can be oxidized by methanotrophic bacteria in oxic water or sediment interfaces before reaching the atmosphere (Ding et al., 2005; Bastviken et al., 2008). Furthermore, more than 90% of diffusive CH_4 fluxes can be consumed by CH_4 oxidation (King, 1990; Ding et al., 2005). In shallow waters, however, aquatic plants rooting in anaerobic soils can directly transport CH_4 from soils to the atmosphere via the aerenchyma system, providing a short-cut for CH_4 to escape from oxidation (Chapter 4; Thomas et al. (1996); Bastviken (2009)). Although this mechanism is known for a number of helophyte species, it has hardly been studied in floating macrophytes. In Chapter 4, we showed for *E. crassipes* that rooting led to up to 4 times higher diffusive CH_4 emission. Additionally, CH_4 emissions are density-dependent, increasing as plant density increases, which may be associated with a combination of plant-enhanced transport and/or plant organic matter production (Chapter 4; Cicerone et al. (1983); Schütz et al. (1989); Whiting et al. (1991); Zhuang et al. (2004)). In addition, CH_4 production is affected by the availability of labile soil organic matter (supplying electron donors such as acetate) and the availability of alternative electron acceptors under anaerobic conditions, such as Fe (III) (Chapter 5; (Yagi and Minami (1990); Dodla et al. (2009))). In Chapter 5, we indeed showed that labile soil organic matter fueled CH_4 production, whereas Fe (III) blocked methanogenesis. Therefore, all above-mentioned factors related to both vegetation and soils should be considered to understand and mitigate CH_4 fluxes from natural and constructed wetlands.

The overall GHG balance

The balance of net CO_2-fluxes and CH_4 emissions can provide an index of a wetland's overall GHG contribution to the atmosphere. Therefore, in order to make constructed wetlands act as net GHG sinks, it is necessary to take measures that minimize CH_4 and CO_2 emissions and maximize CO_2 sequestration (Harpenslager, 2015). Using *E. crassipes*-dominated wetlands for C sequestration under a relatively low density (e.g. 50% coverage) by regular harvest will likely reduce the emission of CH_4 and increase the sequestration of CO_2, especially when roots are prevented from reaching soils by regulating the water level (Chapter 4). Soils with low labile organic matter should be used for wetland construction, as these soils have lower CO_2 emissions than soils with high labile organic matter (Chapter 5; Grogan and Jonasson (2005); Paré and Bedard-Haughn (2013)). In addition, rewetting of drained peat soils should be preferred, as after wetting GHG will be much lower than before wetting due to the slowdown of decomposition rates (Joosten & Clarke, 2002; Harpenslager et al., 2015; Lamers et al., 2015). Although the artificial addition of electron acceptors such as gypsum (sulfate addition) or iron ochre (Fe (III) addition) to a soil can depress CH_4 production and hence reduce CH_4 emissions (Chapter 5; Li et al. (2006); Pangala et al. (2010)), this will simultaneously stimulate decomposition rates and

affect GHG emissions.

Optimizing wetland ecosystem services

After gaining a thorough understanding of the biogeochemical processes that are important for wetland ecosystem services, we are able to design wetland management strategies for managers based on this understanding (Herath, 2004; Euliss et al., 2008). The wetlands studied in the present thesis are constructed and restored mainly for flood abatement (water retention and water storage), soil P mining, wastewater treatment, carbon sequestration by biomass production, or a combination of these services (e.g. in the project Rich Water World, Kwakernaak et al. (2015)). Below, the biogeochemical prerequisites to optimize these four services without negative side effects will be discussed.

Flood abatement

It is predicted that the frequency of severe flooding events will increase in Europe in the future due to heavy precipitation induced by climate changes (Banach et al., 2009; Alfieri et al., 2015), and therefore additional water storage areas are needed during peak discharge periods to prevent flooding of urban areas. Locations that have soils showing low water-extractable P and oxalate-extractable P are strongly preferred for use as water storage areas, as eutrophication can be avoided (Figure 6.1). Otherwise, additional measures such as topsoil removal, or soil P mining by aquatic macrophytes after rewetting, will be required. This is especially important if the surface water is rich in SO_4^{2-} and flooding occurs during summer, as high SO_4^{2-} loads may lead to additional release of P (Lamers et al., 1998; Smolders et al., 2006). In winter the release of nutrients from flooded soils will be much less a problem, as low temperatures decrease microbial activity (Chapter 2; Loeb et al. (2008); Banach et al. (2009)).

Soil P mining

Nature development on former agricultural soils can be a big challenge since they have been heavily fertilized, which results in an extremely nutrient-rich top layer of the soil. As a result, former agricultural soils tend to develop extensive monotonous vegetation consisting of a few high-yield species (Kemmers et al., 2004; Smolders et al., 2008). These vegetation types have little value from an ecological point of view. Low concentrations of nutrients, P in particular, seem to be a prerequisite for long-term coexistence of plant species. This means that the extraction of P from former agricultural soils is crucial for the development of the desired species-rich vegetation types. At the same time, P fertilizers are crucial for sustaining and increasing global food production (Cordell et al., 2009; Van Vuuren et al., 2010). However, P is essentially derived from phosphate rocks and therefore a non-renewable resource, and current phosphate rock reserves

may become exhausted in 50-100 years (Steen, 1998; Cordell, 2008). Therefore, mining soil P has also been proposed as a possible management strategy for alleviating P scarcity in the future (Koopmans et al., 2004). The use of *Azolla* can be beneficial, as N often becomes limiting for the growth of macrophytes, and *Azolla* lives in symbiosis with the dinitrogen-fixing cyanobacterium *Anabaena azollae* preventing N limitation. In addition, regular harvest is necessary for P mining, and harvested biomass can be used as a green fertilizer and animal fodder, thereby achieving P recycling.

Wastewater treatment

Aquatic macrophytes can function as an efficient nutrient filter, but only for low nutrient loads (Chapter 3). At high nutrient loads effective wastewater treatment with aquatic macrophytes can only be achieved in combination with other methods, for instance with a pretreatment, such as flocculation by Fe- and Al-based flocculants or a bio-contact oxidation reactor (Stone et al., 2004; Meers et al., 2006; Wang & Li, 2011). After pretreatment, effluent can be directly discharged into constructed wetlands (Figure 6.1). At low nutrient loads, fast-growing submerged or floating macrophytes can be introduced to constructed wetlands (Chapter 3; Figure 6.1). In Chapter 3, we have shown that at loads $\leq$ 22 mg P m^{-2} d^{-1}, *M. spicatum* is the best option, whereas at loads $\geq$ 22 mg P m^{-2} d^{-1}, *A. filiculoides* removes P more efficiently. Furthermore, the vegetation should be harvested regularly for efficient nutrient removal (Dillaha et al., 1986). An alternative view on wastewater treatment is the use of wastewater as a nutrient source to produce high quality, aquatic macrophyte-based products, such as proteins.

C sequestration

Under aerobic conditions, drained peatlands can emit huge amounts of CO_2 due to aerobic oxidation of organic matter. After rewetting, restoring anaerobic conditions, the decomposition rate of organic matter is slowed down, which may result in C accumulation (Emsens et al., 2015; Harpenslager, 2015). Therefore, rewetting of drained peat soils should be preferred if C sequestration is an important target. Eutrophication of the overlying water, however, can be caused by rewetting of P rich soils (Chapter 2; Van De Riet et al. (2013); Van Diggelen (2015)). As a result, soils showing low water-extractable P and oxalate-extractable P can directly be applied in constructed wetlands. For soils showing high water-extractable and oxalate-extractable P, efficient C sequestration without causing eutrophication can only be achieved in combination with other measures, such as topsoil removal or P mining by *Azolla* after rewetting. Furthermore, peat-forming species such as *Phragmites australis* and *Sphagnum* spp. (Emsens et al., 2015; Harpenslager et al., 2015), should be used to maximize C sequestration from the atmosphere.

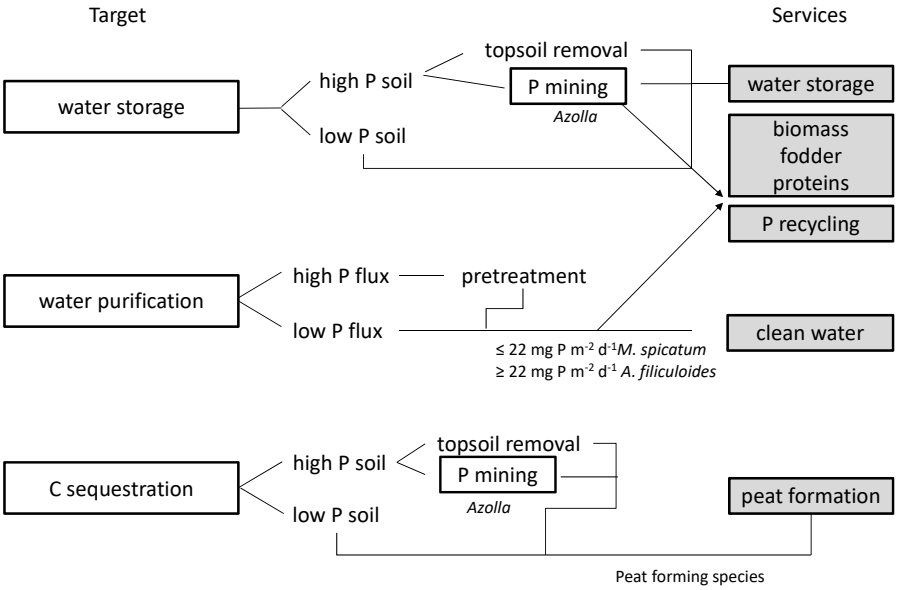


Figure 6.1. Schematic overview of the management strategies to optimize wetland ecosystem services for different targets. See text for further explanation.

General conclusion

This thesis focuses on the key biogeochemical processes in macrophyte-dominated wetlands that determine wetland services. As shown, profound knowledge about these processes is essential for optimizing wetland ecosystem services including flood abatement, soil P mining, wastewater treatment and C sequestration. In addition, this information paves new ways for the development of completely novel types of constructed wetlands, such as cascade systems in which aquatic macrophytes compartments are combined with helophytes and productive infiltration meadows, from which P can be extracted by the use of aquatic macrophytes during temporary flooding (Figure 6.2). Careful selection of plant species and soil types will enable the optimization of multi-service constructed wetlands, in which wastewater is used as a raw material for high quality products (e.g. Aquafarm project, (Durrant et al. (1999))). Although this looks very promising, further research is needed to explore the full potential of such macrophyte-dominated wetland systems.

Figure 6.2. The water purification cascade system constructed at the Radboud Greenhouse outdoor facilities, planted with graminoids (infiltration zone), helophytes (*Iris pseudacorus*), and aquatic macrophytes (*Myriophyllum spicatum*). This system is compared to the adjacent traditional flow system, planted with *Phragmites australis*. Pictures: Y. Tang.

References

Adams, M. S., Guilizzoni, P., Adams, S., 1978. Relationship of dissolved inorganic carbon to macrophyte photosynthesis in some Italian lakes. Limnology and Oceanography 23, 912-919.

Aiken, S. G., Newroth, P. R., Wile, I., 1979. The biology of Canadian weeds. 34. *Myriophyllum spicatum* L. Canadian Journal of Plant Science 59, 201-215.

Alfieri, L., Burek, P., Feyen, L., Forzieri, G., 2015. Global warming increases the frequency of river floods in Europe. Hydrology and Earth System Sciences 19, 2247-2260.

Almeida, R. M., Nóbrega, G. N., Junger, P. C., Figueiredo, A. V., Andrade, A. S., De Moura, C. G. B., Tonetta, D., Oliveira Jr., E. S., Araújo, F., Rust, F., Piñeiro-Guerra, J. M., Mendonça, J., Jurandir R, Medeiros, L. R., Pinheiro, L., Miranda, M., Costa, M. R. A., Melo, M. L., Nobre, R. L. G., Benevides, T., Roland, F., De Klein, J., Barros, N. O., Mendonça, R., Becker, V., Huszar, V. L. M., Kosten, S., 2016. High primary production contrasts with intense carbon emission in a eutrophic tropical reservoir. Frontiers in microbiology 7.

Anderson, N. J., Bennion, H., Lotter, A. F., 2014. Lake eutrophication and its implications for organic carbon sequestration in Europe. Global Change Biology 20, 2741-2751.

Aoyama, I., Nishizaki, H., 1993. Uptake of nitrogen and phosphate, and water purification by water hyacinth *Eichhornia crassipes* (Mart.) Solms. Water Science and Technology 28, 47-53.

Aravind, P., Prasad, M. N. V., Malec, P., Waloszek, A., Strzałka, K., 2009. Zinc protects *Ceratophyllum demersum* L. (free-floating hydrophyte) against reactive oxygen species induced by cadmium. Journal of Trace Elements in Medicine and Biology 23, 50-60.

Attermeyer, K., Flury, S., Jayakumar, R., Fiener, P., Steger, K., Arya, V., Wilken, F., Van Geldern, R., Premke, K., 2016. Invasive floating macrophytes reduce greenhouse gas emissions from a small tropical lake. Scientific reports 6, 20424.

Banach, A. M., Banach, K., Visser, E. J. W., Stępniewska, Z., Smits, A. J. M., Roelofs, J. G. M., Lamers, L. P. M., 2009. Effects of summer flooding on floodplain biogeochemistry in Poland: implications for increased flooding frequency. Biogeochemistry 92, 247-262.

Banik, A., Sen, M., Sen, S. P., 1993. Methane emissions from waterhyacinth-infested freshwater ecosystems. Chemosphere 27, 1539-1552.

Barberis, E., Marsan, F. A., Scalenghe, R., Lammers, A., Schwertmann, U., Edwards, A. C., Maguire, R., Wilson, M. J., Delgado, A., Torrent, J., 1995. European soils overfertilized with phosphorus: Part 1. Basic properties. Fertilizer Research 45, 199-207.

Barko, J. W., Smart, R. M., 1980. Mobilization of sediment phosphorus by submersed freshwater macrophytes. Freshwater Biology 10, 229-238.

Bartlett, K. B., Crill, P. M., Sebacher, D. I., Harriss, R. C., Wilson, J. O., Melack, J. M., 1988. Methane flux from the central Amazonian floodplain. Journal of Geophysical Research: Atmospheres 93, 1571-1582.

Bartlett, M. S., 1947. The use of transformations. Biometrics 3, 39-52.

Bartoń, K., 2015. MuMIn: multi-model inference. R package version 1.15.1. Available at: `http://cran.r-project.org/web/packages/MuMIn/index.html`.

Bastviken, D., 2009. Methane. 1 ed. in: *Encyclopedia of Inland Waters*, (Ed.) G. Likens, Elsevier. Oxford, pp. 783-805.

Bastviken, D., Cole, J., Pace, M., Tranvik, L., 2004. Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate. Global biogeochemical cycles 18, GB4009.

Bastviken, D., Cole, J. J., Pace, M. L., Van de Bogert, M. C., 2008. Fates of methane from different lake habitats: Connecting whole-lake budgets and CH_4 emissions. Journal of Geophysical Research: Biogeosciences 113, G02024.

Bernal, B., Mitsch, W. J., 2012. Comparing carbon sequestration in temperate freshwater wetland communities. Global Change Biology 18, 1636-1647.

Best, M. D., Mantai, K. E., 1978. Growth of *Myriophyllum*: sediment or lake water as the source of nitrogen and phosphorus. Ecology 59, 1075-1080.

Blindow, I., 1992. Decline of charophytes during eutrophication: comparison with angiosperms. Freshwater Biology 28, 9-14.

Blodau, C., 2002. Carbon cycling in peatlands A review of processes and controls. Environmental Reviews 10, 111-134.

Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Cinderby, S., Davidson, E., Dentener, F., Emmett, B., Erisman, J.-W., Fenn, M., Gilliam, F., Nordin, A., Pardo, L., De Vries, W., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological Applications 20, 30-59.

Boers, P. C. M., Van Hese, O., 1988. Phosphorus release from the peaty sediments of the Loosdrecht Lakes (The Netherlands). Water Research 22, 355-363.

Boström, B., Andersen, J. M., Fleischer, S., Jansson, M., 1988. Exchange of phosphorus across the sediment-water interface. in: *Phosphorus in Freshwater Ecosystems*, (Eds.) G. Persson, M. Jansson, Springer Netherlands, pp. 229-244.

Bostrom, B., Jansson, M., Forsberg, C., 1982. Phosphorous release from lake sediments.

Bowden, W. B., 1987. The biogeochemistry of nitrogen in freshwater wetlands. Biogeochemistry 4, 313-348.

Box, R. J., 1986. Quantitative short-term uptake of inorganic phosphate by the *Chara hispida* rhizoid. Plant, Cell & Environment 9, 501-506.

Box, R. J., Boxer, M., Boxer, D., 1985. Compartmentation of PO_4-uptake and ^{32}P efflux in the rhizoid cells of *Chara hispida* L. Biochemie und Physiologie der Pflanzen 180, 551-555.

Bridges, E. M., 1978. World Soils. *2 ed*. Cambridge University Press, Cambridge, England.

Bridgham, S. D., Megonigal, J. P., Keller, J. K., Bliss, N. B., Trettin, C., 2006. The carbon balance of North American wetlands. Wetlands 26, 889-916.

Brisson, J., Chazarenc, F., 2009. Maximizing pollutant removal in constructed wetlands: Should we pay more attention to macrophyte species selection? Science of the Total Environment 407, 3923-3930.

Brix, H., 1999. How 'green'are aquaculture, constructed wetlands and conventional wastewater treatment systems? Water Science and Technology 40, 45-50.

Brix, H., 2003. Plants used in constructed wetlands and their functions. *Proceedings of the First International Seminar on the Use of Aquatic Macrophytes for Wastewater Treatment in Constructed Wetlands*, 8–10 May, Ministerio das Cidades, Ordenamento do Territorio E Ambiente, Lisboa, Portugal. pp. 81-109.

Brix, H., Arias, C. A., 2005. The use of vertical flow constructed wetlands for on-site treatment of domestic wastewater: New Danish guidelines. Ecological Engineering 25, 491-500.

Brix, H., Sorrell, B. K., Lorenzen, B., 2001. Are *Phragmites*-dominated wetlands a net source or net sink of greenhouse gases? Aquatic Botany 69, 313-324.

Brown, S. C., Bedford, B. L., 1997. Restoration of wetland vegetation with transplanted wetland soil: An experimental study. Wetlands 17, 424-437.

Burnham, K. P., Anderson, D. R., 2002. Model selection and multimodel inference: a practical information-theoretic approach. *2 ed*. Springer-Verlag, New York.

Caraco, N., Cole, J., Findlay, S., Wigand, C., 2006. Vascular plants as engineers of oxygen in aquatic systems. Bioscience 56, 219-225.

Caraco, N. F., Cole, J. J., Likens, G. E., 1989. Evidence for sulphate-controlled phosphorus release from sediments of aquatic systems. Nature 341, 316-318.

Carignan, R., Kalff, J., 1980. Phosphorus sources for aquatic weeds: water or sediments? Science 207, 987-989.

Chacon, N., Silver, W. L., Dubinsky, E. A., Cusack, D. F., 2006. Iron reduction and soil phosphorus solubilization in humid tropical forests soils: the roles of labile carbon pools and an electron shuttle compound. Biogeochemistry 78, 67-84.

Chanakya, H. N., Borgaonkar, S., Meena, G., Jagadish, K. S., 1993. Solid-phase biogas production with garbage or water hyacinth. Bioresource Technology 46, 227-231.

Chang, C. C., Cheng, W., 2016. Multiple dietary administrating strategies of water hyacinth (*Eichhornia crassipes*) on enhancing the immune responses and disease resistance of giant freshwater prawn, *Macrobrachium rosenbergii*. Aquaculture Research 47, 140-152.

Chorom, M., Parnian, A., Jaafarzadeh, N., 2012. Nickel removal by the aquatic plant (*Ceratophyllum demersum* L.). International Journal of Environmental Science and Development 3, 372-375.

Cicerone, R. J., Shetter, J. D., Delwiche, C. C., 1983. Seasonal variation of methane flux from a California rice paddy. Journal of Geophysical Research: Oceans 88, 11022-11024.

Clarkson, B. R., Ausseil, A.-G. E., Gerbeaux, P., 2013. Wetland ecosystem services. in: *Ecosystem services in New Zealand: conditions and trends* Manaaki Whenua Press. Lincoln, New Zealand, pp. 192-202.

Conley, D. J., Paerl, H. W., Howarth, R. W., Boesch, D. F., Seitzinger, S. P., Havens, K. E., Lancelot, C., Likens, G. E., 2009. Controlling eutrophication: nitrogen and phosphorus. Science 323, 1014-1015.

Cordell, D., 2008. The Story of Phosphorus: missing global governance of a critical resource. *SENSE Earth Systems Governance* 24-31 August, Amsterdam.

Cordell, D., Drangert, J.-O., White, S., 2009. The story of phosphorus: Global food security and food for thought. Global Environmental Change 19, 292-305.

Costanza, R., d'Arge, R., De Groot, R., Faber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R. V., Paruelo, J., Raskin, R. G., Sutton, P., Van den Belt, M., 1997. The value of the world's ecosystem services and natural capital. Nature 387, 253-260.

Coulthard, T. J., Baird, A. J., Ramirez, J., Waddington, J. M., 2009. Methane dynamics in peat: Importance of shallow peats and a novel reduced-complexity approach for modeling ebullition. in: *Carbon Cycling in Northern Peatlands*, (Eds.) A.J. Baird, L.R. Belyea, X. Comas, A.S. Reeve, L.D. Slater, Vol. 184, American Geophysical Union. Washington, D. C., pp. 173-185.

Cronk, J. K., Fennessy, M. S., 2001. Wetland Plants: Biology and Ecology. Lewis Publishers, Boca Raton, Florida.

Cusell, C., Lamers, L. P. M., Van Wirdum, G., Kooijman, A., 2013. Impacts of water level fluctuation on mesotrophic rich fens: acidification vs. eutrophication. Journal of Applied Ecology 50, 998-1009.

De Leeuw, G., Cohen, L., Frohn, L. M., Geernaert, G., Hertel, O., Jensen, B., Jickells, T., Klein, L., Kunz, G. J., Lund, S., Moerman, M., Müller, F., Pedersen, B., Von Salzen, K., Schlünzen, K. H., Schulz, M., Skjøth, C. A., Sorensen, L.-L., Spokes, L., Tamm, S., Vignati, E., 2001. Atmospheric input of nitrogen into the North Sea: ANICE project overview. Continental Shelf Research 21, 2073-2094.

De Lyon, M. J. H., Roelofs, J. G. M., 1986. Waterplanten in relatie tot waterkwaliteit en bodemgesteldheid. Deel 1 and 2. Laboratorium voor Aquatische Oecologie, Katholieke Universiteit Nijmegen, Nijmegen.

DeBusk, T. A., Ryther, J. H., Hanisak, M. D., Williams, L. D., 1981. Effects of seasonality and plant density on the productivity of some freshwater macrophytes. Aquatic Botany 10, 133-142.

Denny, P., 1987. Mineral cycling by wetland plants-a review. Archiv für Hydrobiologie Beiheft Ergebnisse der Limnologie 27, 1-25.

Denny, P., 1972. Sites of nutrient absorption in aquatic macrophytes. Journal of Ecology 60, 819-829.

Dierberg, F. E., DeBusk, T. A., Jackson, S. D., Chimney, M. J., Pietro, K., 2002. Submerged aquatic vegetation-based treatment wetlands for removing phosphorus from agricultural runoff: response to hydraulic and nutrient loading. Water Research 36, 1409-1422.

Dillaha, T. A., Sherrard, J. H., Lee, D., 1986. Long-term effectiveness and maintenance of vegetative filter strips. Virginia Water Resources Research Center, Virginia Polytechnic Institute and State University, Blacksburg.

Ding, J., Wang, R., Fu, W., Zhang, G., 2001. Water hyacinth in China: Its distribution, problems and control status. *Proceedings of the Second Meeting of the Global Working Group for the Biological and Integrated Control of Water Hyacinth*, 9-12 October 2000, Beijing, China. Australian Centre for International Agricultural Research. pp. 29-32.

Ding, W., Cai, Z., Tsuruta, H., 2005. Plant species effects on methane emissions from freshwater marshes. Atmospheric Environment 39, 3199-3207.

Ding, X., Jiang, J., Wang, Y., Wang, W., Ru, B., 1994. Bioconcentration of cadmium in water hyacinth (*Eichhornia crassipes*) in relation to thiol group content. Environmental Pollution 84, 93-96.

Dodla, S. K., Wang, J. J., Delaune, R. D., Breitenbeck, G., 2009. Carbon gas production under different electron acceptors in a freshwater marsh soil. Chemosphere 76, 517-522.

Driever, S. M., van Nes, E. H., Roijackers, R. M. M., 2005. Growth limitation of *Lemna minor* due to high plant density. Aquatic Botany 81, 245-251.

Drizo, A., Comeau, Y., Forget, C., Chapuis, R. P., 2002. Phosphorus saturation potential: A parameter for estimating the longevity of constructed wetland systems. Environmental Science & Technology 36, 4642-4648.

Durrant, A. E., Scrimshaw, M. D., Stratful, I., Lester, J. N., 1999. Review of the feasibility of recovering phosphate from wastewater for use as a raw material by the phosphate industry. Environmental Technology 20, 749-758.

Ebel, M., Evangelou, M. W. H., Schaeffer, A., 2007. Cyanide phytoremediation by water hyacinths (*Eichhornia crassipes*). Chemosphere 66, 816-823.

Einsele, W., 1938. On the chemical and colloid-chemical reactions in iron-phosphate systems from limnochemical and limnogeological views (Über chemische und kolloidchemische Vorgänge in Eisen-Phosphat-Systemen unter limnochemischen und limnogeologischen Gesichtspunkten). Archiv für Hydrobiologie 33, 361-387.

Ekman, S. R., 1999. Early Pleistocene pollen biostratigraphy in the central North Sea. Review of Palaeobotany and Palynology 105, 171-182.

Emsens, W.-J., Aggenbach, C. J. S., Schoutens, K., Smolders, A. J. P., Zak, D., Van Diggelen, R., 2016. Soil iron content as a predictor of carbon and nutrient mobilization in rewetted fens. PLoS ONE 11, e0153166.

Emsens, W.-J., Aggenbach, C. J. S., Smolders, A. J. P., Van Diggelen, R., 2015. Topsoil removal in degraded rich fens: Can we force an ecosystem reset? Ecological Engineering 77, 225-232.

Erler, D. V., Eyre, B. D., Davison, L., 2008. The contribution of anammox and denitrification to sediment N_2 production in a surface flow constructed wetland. Environmental Science & Technology 42, 9144-9150.

EU, 2016. Commission Implementing Regulation (EU) 2016/1141 of 13 July 2016 adopting a list of invasive alien species of Union concern pursuant to Regulation (EU) No 1143/2014 of the European Parliament and of the Council. `http://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1468477158043&uri=CELEX:32016R1141`.

Euliss, N. H., Smith, L. M., Wilcox, D. A., Browne, B. A., 2008. Linking ecosystem processes with wetland management goals: Charting a course for a sustainable future. Wetlands 28, 553-562.

Finlayson, C. M., Moser, M., 1991. Wetlands. Facts On File Ltd, Oxford.

Forsberg, C., 1964. Phosphorus, a maximum factor in the growth of Characeae. Nature 201, 517-518.

Frolking, S., Roulet, N., Fuglestvedt, J., 2006. How northern peatlands influence the Earth's radiative budget: Sustained methane emission versus sustained carbon sequestration. Journal of Geophysical Research: Biogeosciences 111, G01008.

Furlanetto, L. M., Marinho, C. C., Palma-Silva, C., Albertoni, E. F., Figueiredo-Barros, M. P., Esteves, F. D. A., 2012. Methane levels in shallow subtropical lake sediments: Dependence on the trophic status of the lake and allochthonous input. Limnologica - Ecology and Management of Inland Waters 42, 151-155.

Furnas, M. M. J., 2003. Catchments and corals: terrestrial runoff to the Great Barrier Reef. Australian Institute of Marine Science & CRC Reef Research Centre, Townsville, Australia.

Gale, P. M., Reddy, K. R., Graetz, D. A., 1994. Wetlands and aquatic processes phosphorus retention by wetland soils used for treated wastewater disposal. Journal of Environmental Quality 23, 370-377.

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., Martinelli, L. A., Seitzinger, S. P., Sutton, M. A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320, 889-892.

Geurts, J. J. M., Smolders, A. J. P., Banach, A. M., Van De Graaf, J. P. M., Roelofs, J. G. M., Lamers, L. P. M., 2010. The interaction between decomposition, net N and P mineralization and their mobilization to the surface water in fens. Water Research 44, 3487-3495.

Geurts, J. J. M., Smolders, A. J. P., Verhoeven, J. T. A., Roelofs, J. G. M., Lamers, L. P. M., 2008. Sediment Fe: PO_4 ratio as a diagnostic and prognostic tool for the restoration of macrophyte biodiversity in fen waters. Freshwater Biology 53, 2101-2116.

Geurts, J. J. M., Van De Wouw, P. A. G., Smolders, A. J. P., Roelofs, J. G. M., Lamers, L. P. M., 2011. Ecological restoration on former agricultural soils: Feasibility of *in situ* phosphate fixation as an alternative to top soil removal. Ecological Engineering 37, 1620-1629.

Gorham, E., 1991. Northern peatlands: role in the carbon cycle and probable responses to climatic warming. Ecological Applications 1, 182-195.

Gottschall, N., Boutin, C., Crolla, A., Kinsley, C., Champagne, P., 2007. The role of plants in the removal of nutrients at a constructed wetland treating agricultural (dairy) wastewater, Ontario, Canada. Ecological Engineering 29, 154-163.

Grace, J. B., Wetzel, R. G., 1978. Production biology of Eurasian watermilfoil (*Myriophyllum spicatum* L.): a review. Journal of Aquatic Plant Management 16, 1-11.

Grasshoff, K., Johannsen, H., 1972. A new sensitive and direct method for the automatic determination of ammonia in sea water. ICES Journal of Marine Science 34, 516-521.

Gratwicke, B., Marshall, B. E., 2001. The impact of *Azolla filiculoides* Lam. on animal biodiversity in streams in Zimbabwe. African Journal of Ecology 39, 216-218.

Greenway, M., 2005. The role of constructed wetlands in secondary effluent treatment and water reuse in subtropical and arid Australia. Ecological Engineering 25, 501-509.

Grime, J. P., 1974. Vegetation classification by reference to strategies. Nature 250, 26-31.

Gripp, A. d. R., Marinho, C. C., Sanches, L. F., Petruzzella, A., Esteves, F. d. A., 2013. The role played by aquatic macrophytes regarding CO_2 balance in a tropical coastal lagoon (Cabiúnas Lagoon, Macaé, RJ). Acta Limnologica Brasiliensia 25, 291-301.

Grogan, P., Jonasson, S., 2005. Temperature and substrate controls on intra-annual variation in ecosystem respiration in two subarctic vegetation types. Global Change Biology 11, 465-475.

Gunnars, A., Blomqvist, S., 1997. Phosphate exchange across the sediment-water interface when shifting from anoxic to oxic conditions an experimental comparison of freshwater and brackish-marine systems. Biogeochemistry 37, 203-226.

Gutiérrez, E. L., Ruiz, E. F., Uribe, E. G., Martínez, J. M., 2001. Biomass and productivity of water hyacinth and their application in control programs. *Proceedings of the Second Meeting of the Global Working Group for the Biological and Integrated Control of Water Hyacinth*, 9-12 October 2000, Beijing, China. Australian Centre for International Agricultural Research. pp. 109-119.

Handley, L. L., Raven, J. A., 1992. The use of natural abundance of nitrogen isotopes in plant physiology and ecology. Plant, Cell & Environment 15, 965-985.

Hao, X., Heijnen, J. J., Van Loosdrecht, M. C. M., 2002. Model-based evaluation of temperature and inflow variations on a partial nitrification–ANAMMOX biofilm process. Water Research 36, 4839-4849.

Hao, X., Wang, C., Van Loosdrecht, M. C. M., Hu, Y., 2013. Looking beyond struvite for P-recovery. Environmental Science & Technology 47, 4965-4966.

Harpenslager, S. F., 2015. Growing peat. in: *Aquatic Ecology & Environmental Biology*, Radboud University Nijmegen. Nijmegen, the Netherlands, pp. 175.

Harpenslager, S. F., Lamers, L. P. M., Van Der Heide, T., Roelofs, J. G. M., Smolders, A. J. P., 2016. Harnessing facilitation: Why successful re-introduction of *Stratiotes aloides* requires high densities under high nitrogen loading. Biological Conservation 195, 17-23.

Harpenslager, S. F., Van Den Elzen, E., Kox, M. A. R., Smolders, A. J. P., Ettwig, K. F., Lamers, L. P. M., 2015. Rewetting former agricultural peatlands: Topsoil removal as a prerequisite to avoid strong nutrient and greenhouse gas emissions. Ecological Engineering 84, 159-168.

Harter, R. D., 1969. Phosphorus adsorption sites in soils. Soil Science Society of America Journal 33, 630-632.

Hauck, F. W., 1978. China: recycling of organic wastes in agriculture. Food and Agricultural Organization of the United Nations, Rome.

Heilman, M. A., Carlton, R. G., 2001. Methane oxidation associated with submersed vascular macrophytes and its impact on plant diffusive methane flux. Biogeochemistry 52, 207-224.

Helrich, K., 1990. Official methods of analysis of the Association of Official Analytical Chemists. *15th ed*. AOAC, Arlington, VA.

Henriksen, A., 1965. An automatic method for determining low-level concentrations of phosphates in fresh and saline waters. Analyst 90, 29-34.

Herath, G., 2004. Incorporating community objectives in improved wetland management: the use of the analytic hierarchy process. Journal of Environmental Management 70, 263-273.

Hill, D., 1977. The role of *Anabaena* in the *Azolla-Anabaena* symbiosis. New Phytologist 78, 611-616.

Hill, M. P., 1998. Life history and laboratory host range of Stenopelmus rufinasus, a natural enemy for *Azolla filiculoides* in South Africa. BioControl 43, 215-224.

Holdren, G. C., Armstrong, D. E., 1980. Factors affecting phosphorus release from intact lake sediment cores. Environmental Science & Technology 14, 79-87.

Hooda, P. S., Rendell, A. R., Edwards, A. C., Withers, P. J. A., Aitken, M. N., Truesdale, V. W., 2000. Relating soil phosphorus indices to potential phosphorus release to water. Journal of Environmental Quality 29, 1166-1171.

Houba, V. J. G., Van Der Lee, J. J., Novozamsky, I., Walinga, I., 1989. Soil and plant analysis, a series of syllabi, part 5, soil analysis procedures. Wageningen Agricultural University, Wageningen, The Netherlands.

Howard-Williams, C., 1985. Cycling and retention of nitrogen and phosphorus in wetlands: a theoretical and applied perspective. Freshwater Biology 15, 391-431.

Howarth, R. W., Marino, R., Lane, J., Cole, J. J., 1988. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. Limnology and Oceanography 33, 669-687.

Howarth, R. W., Swaney, D. P., Butler, T. J., Marino, R., 2000. Rapid communication: climatic control on eutrophication of the Hudson River Estuary. Ecosystems 3, 210-215.

IPCC. 2014. Climate change 2014: impacts, adaptation and vulnerability. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC Working Group 2. Cambridge University Press, Port Chester, NY.

Isarankura-Na-Ayudhya, C., Kongpanpee, T., Prabkate, P., Prachayasittikul, V., Tantimongcolwat, T., 2007. Appropriate technology for the bioconversion of water hyacinth (*Eichhornia crassipes*) to liquid ethanol: future prospects for community strengthening and sustainable development. EXCLI Journal 6, 167-176.

Jacinthe, P.-A., Lal, R., Kimble, J. M., 2002. Carbon budget and seasonal carbon dioxide emission from a central Ohio Luvisol as influenced by wheat residue amendment. Soil and Tillage Research 67, 147-157.

Jäger, N., Stange, C. F., Ludwig, B., Flessa, H., 2011. Emission rates of N_2O and CO_2 from soils with different organic matter content from three long-term fertilization experiments-a laboratory study. Biology and Fertility of Soils 47, 483-494.

Jampeetong, A., Brix, H., Kantawanichkul, S., 2012. Effects of inorganic nitrogen forms on growth, morphology, nitrogen uptake capacity and nutrient allocation of four tropical aquatic macrophytes (*Salvinia cucullata*, *Ipomoea aquatica*, *Cyperus involucratus* and *Vetiveria zizanioides*). Aquatic Botany 97, 10-16.

Jensen, H. S., Kristensen, P., Jeppesen, E., Skytthe, A., 1992. Iron: phosphorus ratio in surface sediment as an indicator of phosphate release from aerobic sediments in shallow lakes. Hydrobiologia 235, 731-743.

Jing, S. R., Lin, Y. F., Lee, D. Y., Wang, T. W., 2001. Nutrient removal from polluted river water by using constructed wetlands. Bioresource Technology 76, 131-135.

Joosten, H., Clarke, D., 2002. Wise use of peatlands. International Mire Conservation Group, International Peat Society, Jyväskylä, Finland.

Kadlec, R. H., Wallace, S. D., 2008. Treatment wetlands. *2nd ed*. CRC press, Boca Raton, Florida.

Kamphake, L. J., Hannah, S. A., Cohen, J. M., 1967. Automated analysis for nitrate by hydrazine reduction. Water Research 1, 205-216.

Kantawanichkul, S., Kladprasert, S., Brix, H., 2009. Treatment of high-strength wastewater in tropical vertical flow constructed wetlands planted with *Typha angustifolia* and *Cyperus involucratus*. Ecological Engineering 35, 238-247.

Kaseva, M. E., 2004. Performance of a sub-surface flow constructed wetland in polishing pre-treated wastewater-a tropical case study. Water Research 38, 681-687.

Kemmers, R. H., Beltman, B., Grootjans, A. P., Jansen, A. J. M., Kooijman, G., Schipper, P. C., 2004. Voorkomen en bestrijden van Pitrusdominantie in natte schraallanden. Alterra-rapport Pitrus, Alterra, Wageningen, the Netherlands.

Keskinkan, O., Goksu, M. Z. L., Basibuyuk, M., Forster, C. F., 2004. Heavy metal adsorption properties of a submerged aquatic plant (*Ceratophyllum demersum*). Bioresource Technology 92, 197-200.

Khalid, R. A., Patrick, W. H., DeLaune, R. D., 1977. Phosphorus sorption characteristics of flooded soils. Soil Science Society of America Journal 41, 305-310.

King, G. M., 1990. Dynamics and controls of methane oxidation in a Danish wetland sediment. FEMS Microbiology Letters 74, 309-323.

Knight, R. L., Gu, B., Clarke, R. A., Newman, J. M., 2003. Long-term phosphorus removal in Florida aquatic systems dominated by submerged aquatic vegetation. Ecological Engineering 20, 45-63.

Konnerup, D., Koottatep, T., Brix, H., 2009. Treatment of domestic wastewater in tropical, subsurface flow constructed wetlands planted with *Canna* and *Heliconia*. Ecological Engineering 35, 248-257.

Koopmans, G. F., Chardon, W. J., Ehlert, P. A. I., Dolfing, J., Suurs, R. A. A., Oenema, O., Van Riemsdijk, W. H., 2004. Phosphorus availability for plant uptake in a phosphorus-enriched noncalcareous sandy soil. Journal of Environmental Quality 33, 965-975.

Kosten, S., Huszar, V. L. M., Bécares, E., Costa, L. S., Van Donk, E., Hansson, L. A., Jeppesen, E., Kruk, C., Lacerot, G., Mazzeo, N., De Meester, L., Moss, B., Lürling, M., Nõges, T., Romo, S., Scheffer, M., 2012. Warmer climates boost cyanobacterial dominance in shallow lakes. Global Change Biology 18, 118-126.

Kosten, S., Piñeiro, M., De Goede, E., De Klein, J., Lamers, L. P. M., Ettwig, K., 2016. Fate of methane in aquatic systems dominated by free-floating plants. Water Research 104, 200-207.

Kronvang, B., Jeppesen, E., Conley, D. J., Søndergaard, M., Larsen, S. E., Ovesen, N. B., Carstensen, J., 2005. Nutrient pressures and ecological responses to nutrient loading reductions in Danish streams, lakes and coastal waters. Journal of Hydrology 304, 274-288.

Kufel, L., Kufel, I., 2002. *Chara* beds acting as nutrient sinks in shallow lakes-a review. Aquatic Botany 72, 249-260.

Küsel, K., Wagner, C., Trinkwalter, T., Gößner, A. S., Bäumler, R., Drake, H. L., 2002. Microbial reduction of Fe (III) and turnover of acetate in Hawaiian soils. FEMS Microbiology Ecology 40, 73-81.

Kwakernaak, C., Jansen, P. C., Kempen, M., Smolders, F., Rheenen, H., 2015. A smart solution for flooding, drought and water pollution. Water Matters: Knowledge Journal for Water Professionals, 44-47.

Lamers, L. P. M., Falla, S. J., Samborska, E. M., Van Dulken, I. A. R., Van Hengstum, G., Roelofs, J. G. M., 2002a. Factors controlling the extent of eutrophication and toxicity in sulfate-polluted freshwater wetlands. Limnology and Oceanography 47, 585-593.

Lamers, L. P. M., Loeb, R., Antheunisse, A. M., Miletto, M., Lucassen, E. C. H. E. T., Boxman, A. W., Smolders, A. J. P., Roelofs, J. G. M., 2006. Biogeochemical constraints on the ecological rehabilitation of wetland vegetation in river floodplains. Hydrobiologia 565, 165-186.

Lamers, L. P. M., Smolders, A. J. P., Roelofs, J. G. M., 2002b. The restoration of fens in the Netherlands. Hydrobiologia 478, 107-130.

Lamers, L. P. M., Ten Dolle, G. E., Van Den Berg, S. T. G., Van Delft, S. P. J., Roelofs, J. G. M., 2001. Differential responses of freshwater wetland soils to sulphate pollution. Biogeochemistry 55, 87-101.

Lamers, L. P. M., Tomassen, H. B. M., Roelofs, J. G. M., 1998. Sulfate-induced eutrophication and phytotoxicity in freshwater wetlands. Environmental Science & Technology 32, 199-205.

Lamers, L. P. M., Vile, M. A., Grootjans, A. P., Acreman, M. C., Van Diggelen, R., Evans, M. G., Richardson, C. J., Rochefort, L., Kooijman, A. M., Roelofs, J. G. M., Smolders, A. J. P., 2015. Ecological restoration of rich fens in Europe and North America: from trial and error to an evidence-based approach. Biological Reviews 90, 182-203.

Le, C., Zha, Y., Li, Y., Sun, D., Lu, H., Yin, B., 2010. Eutrophication of lake waters in China: cost, causes, and control. Environmental Management 45, 662-668.

Li, C., Salas, W., DeAngelo, B., Rose, S., 2006. Assessing alternatives for mitigating net greenhouse gas emissions and increasing yields from rice production in China over the next twenty years. Journal of Environmental Quality 35, 1554-1565.

Li, X., Mander, Ü., Ma, Z., Jia, Y., 2009. Water quality problems and potential for wetlands as treatment systems in the Yangtze River Delta, China. Wetlands 29, 1125-1132.

Liikanen, A., Murtoniemi, T., Tanskanen, H., Väisänen, T., Martikainen, P. J., 2002. Effects of temperature and oxygen availability on greenhouse gas and nutrient dynamics in sediment of a eutrophic mid-boreal lake. Biogeochemistry 59, 269-286.

Lin, Y. F., Jing, S. R., Lee, D. Y., 2003. The potential use of constructed wetlands in a recirculating aquaculture system for shrimp culture. Environmental Pollution 123, 107-113.

Lin, Y. F., Jing, S. R., Wang, T. W., Lee, D. Y., 2002. Effects of macrophytes and external carbon sources on nitrate removal from groundwater in constructed wetlands. Environmental Pollution 119, 413-420.

Loeb, R., Lamers, L. P., Roelofs, J. G., 2008. Prediction of phosphorus mobilisation in inundated floodplain soils. Environmental Pollution 156, 325-331.

Loeb, R., Van Daalen, E., Lamers, L. P. M., Roelofs, J. G. M., 2007. How soil characteristics and water quality influence the biogeochemical response to flooding in riverine wetlands. Biogeochemistry 85, 289-302.

Lombardo, P., Cooke, G. D., 2003. *Ceratophyllum demersum*–phosphorus interactions in nutrient enriched aquaria. Hydrobiologia 497, 79-90.

Lovley, D. R., Phillips, E. J. P., 1988. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. Applied and Environmental Microbiology 54, 1472-1480.

Ludwig, B., Geisseler, D., Michel, K., Joergensen, R. G., Schulz, E., Merbach, I., Raupp, J., Rauber, R., Hu, K., Niu, L., Liu, X., 2011. Effects of fertilization and soil management on crop yields and carbon stabilization in soils. A review. Agronomy for sustainable development 31, 361-372.

Lumpkin, T. A., Plucknett, D. L., 1982. *Azolla* as a green manure: use and management in crop production. Westview Press, Boulder, Colorado.

Malik, A., 2007. Environmental challenge *vis* a *vis* opportunity: the case of water hyacinth. Environment International 33, 122-138.

Mandi, L., 1994. Marrakesh wastewater purification experiment using vascular aquatic plants *Eichhornia crassipes* and *Lemna gibba*. Water Science and Technology 29, 283-287.

Masifwa, W. F., Twongo, T., Denny, P., 2001. The impact of water hyacinth, *Eichhornia crassipes* (Mart) Solms on the abundance and diversity of aquatic macroinvertebrates along the shores of northern Lake Victoria, Uganda. Hydrobiologia 452, 79-88.

McLatchey, G. P., Reddy, K. R., 1998. Regulation of organic matter decomposition and nutrient release in a wetland soil. Journal of Environmental Quality 27, 1268-1274.

Medvedeff, C. A., Inglett, K. S., Inglett, P. W., 2014. Evaluation of direct and indirect phosphorus limitation of methanogenic pathways in a calcareous subtropical wetland soil. Soil Biology and Biochemistry 69, 343-345.

Meers, E., Rousseau, D. P. L., Lesage, E., Demeersseman, E., Tack, F. M. G., 2006. Physico-chemical P removal from the liquid fraction of pig manure as an intermediary step in manure processing. Water, air, and soil pollution 169, 317-330.

Melchior, M., 1997. Lake restoration using mechanical, chemical and biological control strategies for Eurasian water milfoil (*Myriophyllum spicatum*). Restoration and Reclamation Review 2.

Méthy, M., Alpert, P., Roy, J., 1990. Effects of light quality and quantity on growth of the clonal plant *Eichhornia crassipes*. Oecologia 84, 265-271.

Millennium Ecosystem Assessment. 2005. Ecosystems and human well-being: wetlands and water. World Resources Institute, Washington, DC.

Mitsch, W. J., Bernal, B., Nahlik, A. M., Mander, Ü., Zhang, L., Anderson, C. J., Jørgensen, S. E., Brix, H., 2013. Wetlands, carbon, and climate change. Landscape Ecology 28, 583-597.

Mitsch, W. J., Gosselink, J. G., 2000. The value of wetlands: importance of scale and landscape setting. Ecological Economics 35, 25-33.

Mitsch, W. J., Gosselink, J. G., 2007. Wetlands. *4th ed*. John Wiley & Sons, New York.

Mitsch, W. J., Zhang, L., Anderson, C. J., Altor, A. E., Hernandez, M. E., 2005. Creating riverine wetlands: Ecological succession, nutrient retention, and pulsing effects. Ecological Engineering 25, 510-527.

Mjelde, M., Faafeng, B., 1997. *Ceratophyllum demersum* hampers phytoplankton development in some small Norwegian lakes over a wide range of phosphorus concentrations and geographical latitude. Freshwater Biology 37, 355-365.

Moore, A., Reddy, K. R., 1994. Role of Eh and pH on phosphorus geochemistry in sediments of Lake Okeechobee, Florida. Journal of Environmental Quality 23, 955-964.

Moore, A. W., 1969. *Azolla*: Biology and agronomic significance. The Botanical Review 35, 17-34.

Moore, T. R., Roulet, N. T., Waddington, J. M., 1998. Uncertainty in predicting the effect of climatic change on the carbon cycling of Canadian peatlands. Climatic Change 40, 229-245.

Moreno-Mateos, D., Power, M. E., Comín, F. A., Yockteng, R., 2012. Structural and functional loss in restored wetland ecosystems. PLoS Biology 10, e1001247.

Mortimer, C. H., 1941. The exchange of dissolved substances between mud and water in lakes. Journal of Ecology 29, 280-329.

Muramoto, S., Oki, Y., 1983. Removal of some heavy metals from polluted water by water hyacinth (*Eichhornia crassipes*). Bulletin of Environmental Contamination and Toxicology 30, 170-177.

Myhre, G., Shindell, D., Bréon, F.-M., Collins, W., Fuglestvedt, J., Huang, J., Koch, D., Lamarque, J.-F., Lee, D., Mendoza, B., Nakajima, T., Robock, A., Stephens, G., Takemura, T., Zhang, H., 2013. Anthropogenic and natural radiative forcing. in: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, (Eds.) T.F. Stocker, D. Qin, G.-K. Plattner, M.M.B. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, P.M. Midgley, Cambridge University Press. Cambridge, U.K., pp. 659-740.

Nahlik, A. M., Mitsch, W. J., 2006. Tropical treatment wetlands dominated by free-floating macrophytes for water quality improvement in Costa Rica. Ecological Engineering 28, 246-257.

Natchimuthu, S., Selvam, B. P., Bastviken, D., 2014. Influence of weather variables on methane and carbon dioxide flux from a shallow pond. Biogeochemistry 119, 403-413.

Navid, D., 1989. The international law of migratory species: the Ramsar Convention. Natural Resources Journal 29, 1001-1016.

Nichols, D. S., Keeney, D. R., 1976. Nitrogen nutrition of *Myriophyllum spicatum*: variation of plant tissue nitrogen concentration with season and site in Lake Wingra. Freshwater Biology 6, 137-144.

Nichols, S. A., Shaw, B. H., 1986. Ecological life histories of the three aquatic nuisance plants, *Myriophyllum spicatum*, *Potamogeton crispus* and *Elodea canadensis*. Hydrobiologia 131, 3-21.

Nuttall, P. M., 1985. Uptake of phosphorus and nitrogen by *Myriophyllum aquaticum* (Velloza) Verd. Growing in a wastewater treatment system. Marine & Freshwater Research 36, 493-507.

OECD. 1996. Guidelines for aid agencies for improved conservation and sustainable use of tropical and sub-tropical wetlands. Organization for Economic Co-operation and Development, Paris, France.

Olsen, S. R., Cole, C. V., Watanabe, F. S., Dean, L. A., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Department of Agriculture Circular No 939. US Government Print Office, Washington, D.C.

Ornes, W. H., Sajwan, K. S., 1993. Cadmium accumulation and bioavailability in Coontail (*Ceratophyllum demersum* L.) plants. Water, air, and soil pollution 69, 291-300.

Pangala, S. R., Reay, D. S., Heal, K. V., 2010. Mitigation of methane emissions from constructed farm wetlands. Chemosphere 78, 493-499.

Pant, H. K., Reddy, K. R., 2003. Potential internal loading of phosphorus in a wetland constructed in agricultural land. Water Research 37, 965-972.

Paré, M. C., Bedard-Haughn, A., 2013. Soil organic matter quality influences mineralization and GHG emissions in cryosols: a field-based study of sub- to high Arctic. Global Change Biology 19, 1126-1140.

Patrick Jr., W. H., Khalid, R. A., 1974. Phosphate release and sorption by soils and sediments: effect of aerobic and anaerobic conditions. Science 186, 53-55.

Peixoto, R. B., Marotta, H., Bastviken, D., Enrich-Prast, A., 2016. Floating aquatic macrophytes can substantially offset open water CO_2 emissions from tropical floodplain lake ecosystems. Ecosystems 19, 724-736.

Peters, G. A., Meeks, J. C., 1989. The *Azolla-Anabaena* symbiosis: basic biology. Annual Review of Plant Physiology and Plant Molecular Biology 40, 193-210.

Petzet, S., Peplinski, B., Cornel, P., 2012. On wet chemical phosphorus recovery from sewage sludge ash by acidic or alkaline leaching and an optimized combination of both. Water Research 46, 3769-3780.

Pillai, P. K., Premalatha, S., Rajamony, S., 2005. *Azolla*: a sustainable feed for livestock. Leisa Magazine, 26-27.

Pinto-Coelho, R. M., Greco, M. K. B., 1999. The contribution of water hyacinth (*Eichhornia crassipes*) and zooplankton to the internal cycling of phosphorus in the eutrophic Pampulha Reservoir, Brazil. Hydrobiologia 411, 115-127.

Poffenbarger, H. J., Needelman, B. A., Megonigal, J. P., 2011. Salinity influence on methane emissions from tidal marshes. Wetlands 31, 831-842.

Polomski, R. F., Taylor, M. D., Bielenberg, D. G., Bridges, W. C., Klaine, S. J., Whitwell, T., 2009. Nitrogen and phosphorus remediation by three floating aquatic macrophytes in greenhouse-based laboratory-scale subsurface constructed wetlands. Water, air, and soil pollution 197, 223-232.

Polprasert, C., Khatiwada, N. R., 1998. An integrated kinetic model for water hyacinth ponds used for wastewater treatment. Water Research 32, 179-185.

Ponnamperuma, F. N., 1984. Effects of flooding on soils. in: *Flooding and plant growth*, (Ed.) T.T. Kozlowski, Academic Press, INC. Orlando, Florida, pp. 9-45.

Pretty, J. N., Mason, C. F., Nedwell, D. B., Hine, R. E., Leaf, S., Dils, R., 2003. Environmental costs of freshwater eutrophication in England and Wales. Environmental Science & Technology 37, 201-208.

Ramulu, U. S. S., Pratt, P. F., Page, A. L., 1967. Phosphorus fixation by soils in relation to extractable iron oxides and mineralogical composition. Soil Science Society of America Journal 31, 193-196.

Ran, N., Agami, M., Oron, G., 2004. A pilot study of constructed wetlands using duckweed (*Lemna gibba* L.) for treatment of domestic primary effluent in Israel. Water Research 38, 2241-2248.

Reddy, K., DeBusk, W., 1991. Decomposition of water hyacinth detritus in eutrophic lake water. Hydrobiologia 211, 101-109.

Reddy, K., DeBusk, W., 1985. Growth characteristics of aquatic macrophytes cultured in nutrient-enriched water: II. Azolla, Duckweed, and Salvinia. Economic Botany 39, 200-208.

Reddy, K., Patrick, W., Broadbent, F., 1984. Nitrogen transformations and loss in flooded soils and sediments. Critical Reviews in Environmental Science and Technology 13, 273-309.

Reddy, K., Tucker, J., 1983. Productivity and nutrient uptake of water hyacinth, Eichhornia crassipes I. Effect of nitrogen source. Economic Botany 37, 237-247.

Reddy, K. R., Agami, M., Tucker, J. C., 1989. Influence of nitrogen supply rates on growth and nutrient storage by water hyacinth (*Eichhornia crassipes*) plants. Aquatic Botany 36, 33-43.

Reddy, K. R., Agami, M., Tucker, J. C., 1990. Influence of phosphorus on growth and nutrient storage by water hyacinth (*Eichhornia crassipes* (Mart.) Solms) plants. Aquatic Botany 37, 355-365.

Reddy, K. R., D'Angelo, E. M., 1994. Soil processes regulating water quality in wetlands. in: *Global wetlands: Old world and new*, (Ed.) W.J. Mitsch, Elsevier. Amsterdam, The Netherlands, pp. 309-324.

Reddy, K. R., DeLaune, R. D., 2008. Biogeochemistry of wetlands: science and applications. CRC press.

Richardson, C. J., 1985. Mechanisms controlling phosphorus retention capactiy in freshwater wetlands. Science 228, 1424-1427.

Rodrigo, M. A., Rojo, C., Álvarez-Cobelas, M., Cirujano, S., 2007. *Chara hispida* beds as a sink of nitrogen: Evidence from growth, nitrogen uptake and decomposition. Aquatic Botany 87, 7-14.

Roelofs, J. G. M., 1991. Inlet of alkaline river water into peaty lowlands: effects on water quality and *Stratiotes aloides* L. stands. Aquatic Botany 39, 267-293.

Roy, J. W., Bickerton, G., 2014. Elevated dissolved phosphorus in riparian groundwater along gaining urban streams. Environmental Science & Technology 48, 1492-1498.

Sanyahumbi, D., Duncan, J. R., Zhao, M., Van Hille, R., 1998. Removal of lead from solution by the non-viable biomass of the water fern *Azolla filiculoides*. Biotechnology Letters 20, 745-747.

Scholz, M., Xu, J., 2002. Performance comparison of experimental constructed wetlands with different filter media and macrophytes treating industrial wastewater contaminated with lead and copper. Bioresource Technology 83, 71-79.

Schütz, H., Seiler, W., Conrad, R., 1989. Processes involved in formation and emission of methane in rice paddies. Biogeochemistry 7, 33-53.

Sibrell, P. L., Montgomery, G. A., Ritenour, K. L., Tucker, T. W., 2009. Removal of phosphorus from agricultural wastewaters using adsorption media prepared from acid mine drainage sludge. Water Research 43, 2240-2250.

Singh, S. N., Kulshreshtha, K., Agnihotri, S., 2000. Seasonal dynamics of methane emission from wetlands. Chemosphere-Global Change Science 2, 39-46.

Smil, V., 2000. Phosphorus in the environment: natural flows and human interferences. Annual review of energy and the environment 25, 53-88.

Smith, C. S., Adams, M. S., 1986. Phosphorus transfer from sediments by *Myriophyllum spicaturn*. Limnology and Oceanography 31, 1312-1321.

Smith, C. S., Barko, J. W., 1990. Ecology of Eurasian watermilfoil. Journal of Aquatic Plant Management 28, 55-64.

Smith, V. H., 2003. Eutrophication of freshwater and coastal marine ecosystems a global problem. Environmental Science and Pollution Research 10, 126-139.

Smolders, A. J. P., Lamers, L. P. M., Lucassen, E. C. H. E. T., Van Der Velde, G., Roelofs, J. G. M., 2006. Internal eutrophication: How it works and what to do about it-a review. Chemistry and Ecology 22, 93-111.

Smolders, A. J. P., Lamers, L. P. M., Moonen, M., Zwaga, K., Roelofs, J. G. M., 2001. Controlling phosphate release from phosphate-enriched sediments by adding various iron compounds. Biogeochemistry 54, 219-228.

Smolders, A. J. P., Lucassen, E. C. H. E. T., Bobbink, R., Roelofs, J. G. M., Lamers, L. P. M., 2010. How nitrate leaching from agricultural lands provokes phosphate eutrophication in groundwater fed wetlands: the sulphur bridge. Biogeochemistry 98, 1-7.

Smolders, A. J. P., Lucassen, E. C. H. E. T., Van Der Aalst, M., Lamers, L. P. M., Roelofs, J. G. M., 2008. Decreasing the abundance of *Juncus effusus* on former agricultural lands with noncalcareous sandy soils: possible effects of liming and soil removal. Restoration Ecology 16, 240-248.

Speelman, E. N., Van Kempen, M. M. L., Barke, J., Brinkhuis, H., Reichart, G. J., Smolders, A. J. P., Roelofs, J. G. M., Sangiorgi, F., De Leeuw, J. W., Lotter, A. F., Damsté, J. S. S., 2009. The Eocene Arctic *Azolla* bloom: environmental conditions, productivity and carbon drawdown. Geobiology 7, 155-170.

Sperber, J. I., 1958. Release of phosphate from soil minerals by hydrogen sulphide. Nature 181, 934.

Steen, I., 1998. Phosphorus availability in the 21st century: Management of a non-renewable resource. Phosphorus Potassium 217, 25-31.

Stocker, T. F., Qin, D., Plattner, G.-K., Tignor, M. M. B., Allen, S. K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P. M., 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, U.K.

Stone, K. C., Poach, M. E., Hunt, P. G., Reddy, G. B., 2004. Marsh-pond-marsh constructed wetland design analysis for swine lagoon wastewater treatment. Ecological Engineering 23, 127-133.

Ström, L., Mastepanov, M., Christensen, T. R., 2005. Species-specific effects of vascular plants on carbon turnover and methane emissions from wetlands. Biogeochemistry 75, 65-82.

Swift, M. J., Heal, O. W., Anderson, J. M., 1979. Decomposition in terrestrial ecosystems. Univeristy of California Press, Berkeley, CA.

Tang, Y., Harpenslager, S. F., Van Kempen, M. M. L., Verbaarschot, E. J. H., Loeffen, L. M. J. M., Roelofs, J. G. M., Smolders, A. J. P., Lamers, L. P. M., 2016a. Aquatic macrophytes can be used for wastewater polishing, but not for purification in constructed wetlands. Biogeosciences Discussions.

Tang, Y., Van Kempen, M. M. L., Van Der Heide, T., Manschot, J. J. A., Roelofs, J. G. M., Lamers, L. P. M., Smolders, A. J. P., 2016b. A tool for easily predicting short-term phosphorus mobilization from flooded soils. Ecological Engineering 94, 1-6.

Tanner, C. C., 1996. Plants for constructed wetland treatment systems-A comparison of the growth and nutrient uptake of eight emergent species. Ecological Engineering 7, 59-83.

Téllez, T. R., López, E. M. D. R., Granado, G. L., Pérez, E. A., López, R. M., Guzmán, J. M. S., 2008. The water hyacinth, *Eichhornia crassipes*: an invasive plant in the Guadiana River Basin (Spain). Aquatic Invasions 3, 42-53.

Thomas, K. L., Benstead, J., Davies, K. L., Lloyd, D., 1996. Role of wetland plants in the diurnal control of CH_4 and CO_2 fluxes in peat. Soil Biology and Biochemistry 28, 17-23.

Toetz, D. W., 1974. Uptake and translocation of ammonia by freshwater hydrophytes. Ecology 55, 199-201.

Tokida, T., Miyazaki, T., Mizoguchi, M., Nagata, O., Takakai, F., Kagemoto, A., Hatano, R., 2007. Falling atmospheric pressure as a trigger for methane ebullition from peatland. Global biogeochemical cycles 21, GB2003.

Tomassen, H. B. M., Smolders, A. J. P., Limpens, J., Lamers, L. P. M., Roelofs, J. G. M., 2004. Expansion of invasive species on ombrotrophic bogs: desiccation or high N deposition? Journal of Applied Ecology 41, 139-150.

Van Bodegom, P. M., Scholten, J. C. M., Stams, A. J. M., 2004. Direct inhibition of methanogenesis by ferric iron. FEMS Microbiology Ecology 49, 261-268.

Van De Riet, B. P., Hefting, M. M., Verhoeven, J. T. A., 2013. Rewetting drained peat meadows: risks and benefits in terms of nutrient release and greenhouse gas exchange. Water, Air, & Soil Pollution 224, 1440-1452.

Van Der Gon, H. A. C. D., Neue, H.-U., 1995. Influence of organic matter incorporation on the methane emission from a wetland rice field. Global biogeochemical cycles 9, 11-22.

Van Der Star, W. R. L., Abma, W. R., Blommers, D., Mulder, J.-W., Tokutomi, T., Strous, M., Picioreanu, C., Van Loosdrecht, M. C. M., 2007. Startup of reactors for anoxic ammonium oxidation: Experiences from the first full-scale anammox reactor in Rotterdam. Water Research 41, 4149-4163.

Van Diggelen, J. M. H., 2015. Human impact on peatlands: from biogeochemical issues towards sustainable and use options. in: *Aquatic Ecology* & *Environmental Biology*, Radboud University Nijmegen. Nijmegen, the Netherlands, pp. 139.

Van Dijk, J., Stroetenga, M., Bos, L., Van Bodegom, P. M., Verhoef, H. A., Aerts, R., 2004. Restoring natural seepage conditions on former agricultural grasslands does not lead to reduction of organic matter decomposition and soil nutrient dynamics. Biogeochemistry 71, 317-337.

Van Dongen, U., Jetten, M. S. M., Van Loosdrecht, M. C. M., 2001. The SHARON® Anammox® process for treatment of ammonium rich wastewater. Water Science and Technology 44, 153-160.

Van Kempen, M. M. L., 2013. *Azolla* on top of the world: an ecophysiological study of floating fairy moss and its potential role in ecosystem services related to climate change. in: *Aquatic Ecology & Environmental Biology*, Radboud University Nijmegen. Nijmegen, the Netherlands, pp. 165.

Van Kempen, M. M. L., Smolders, A. J. P., Bögemann, G. M., Lamers, L. P. M., Roelofs, J. G. M., 2016. Interacting effects of atmospheric CO_2 enrichment and solar radiation on growth of the aquatic fern *Azolla filiculoides*. Freshwater Biology 61, 596-606.

Van Kempen, M. M. L., Smolders, A. J. P., Bögemann, G. M., Lamers, L. P. M., Visser, E. J. W., Roelofs, J. G. M., 2013. Responses of the *Azolla filiculoides* Stras.-*Anabaena azollae* Lam. association to elevated sodium chloride concentrations: Amino acids as indicators for salt stress and tipping point. Aquatic Botany 106, 20-28.

Van Kempen, M. M. L., Smolders, A. J. P., Lamers, L. P. M., Roelofs, J. G. M., 2012. Micro-halocline enabled nutrient recycling may explain extreme *Azolla* event in the Eocene Arctic Ocean. PLoS ONE 7, e50159.

Van Loosdrecht, M. C. M., Jetten, M. S. M., 1998. Microbiological conversions in nitrogen removal. Water Science and Technology 38, 1-7.

Van Soest, P. J., Robertson, J. B., Lewis, B. A., 1991. Symposium: carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. Journal of Dairy Science 74, 3583-3597.

Van Vuuren, D. P., Bouwman, A. F., Beusen, A. H. W., 2010. Phosphorus demand for the 1970-2100 period: A scenario analysis of resource depletion. Global Environmental Change 20, 428-439.

Verhoeven, J. T. A., Arheimer, B., Yin, C., Hefting, M. M., 2006. Regional and global concerns over wetlands and water quality. Trends in Ecology & Evolution 21, 96-103.

Villamagna, A. M., Murphy, B. R., 2010. Ecological and socio-economic impacts of invasive water hyacinth (*Eichhornia crassipes*): a review. Freshwater Biology 55, 282-298.

Vrhovšek, D., Kukanja, V., Bulc, T., 1996. Constructed wetland (CW) for industrial waste water treatment. Water Research 30, 2287-2292.

Vymazal, J., 2011. Plants used in constructed wetlands with horizontal subsurface flow: a review. Hydrobiologia 674, 133-156.

Vymazal, J., 2007. Removal of nutrients in various types of constructed wetlands. Science of the Total Environment 380, 48-65.

Wang, L., Li, T., 2011. Anaerobic ammonium oxidation in constructed wetlands with bio-contact oxidation as pretreatment. Ecological Engineering 37, 1225-1230.

Weier, K. L., Doran, J. W., Power, J. F., Walters, D. T., 1993. Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. Soil Science Society of America Journal 57, 66-72.

West, R. G., 1953. The occurrence of *Azolla* in British interglacial deposits. New Phytologist 52, 267-272.

Wetzel, R. G., 2001. Limnology: lake and river ecosystems. *3rd ed*. Academic Press, San Diego, California.

Whiting, G. J., Chanton, J. P., 2001. Greenhouse carbon balance of wetlands: methane emission versus carbon sequestration. Tellus B 53, 521-528.

Whiting, G. J., Chanton, J. P., Bartlett, D. S., Happell, J. D., 1991. Relationships between CH_4 emission, biomass, and CO_2 exchange in a subtropical grassland. Journal of Geophysical Research: Atmospheres 96, 13067-13071.

Wilson, J. R., Holst, N., Rees, M., 2005. Determinants and patterns of population growth in water hyacinth. Aquatic Botany 81, 51-67.

Wolf, K. L., Ahn, C., Noe, G. B., 2011. Microtopography enhances nitrogen cycling and removal in created mitigation wetlands. Ecological Engineering 37, 1398-1406.

Wu, S., Kuschk, P., Brix, H., Vymazal, J., Dong, R., 2014. Development of constructed wetlands in performance intensifications for wastewater treatment: A nitrogen and organic matter targeted review. Water Research 57, 40-55.

Xie, Y., Wen, M., Yu, D., Li, Y., 2004. Growth and resource allocation of water hyacinth as affected by gradually increasing nutrient concentrations. Aquatic Botany 79, 257-266.

Yagi, K., Minami, K., 1990. Effect of organic matter application on methane emission from some Japanese paddy fields. Soil Science and Plant Nutrition 36, 599-610.

Yoshida, N., Iguchi, H., Yurimoto, H., Murakami, A., Sakai, Y., 2014. Aquatic plant surface as a niche for methanotrophs. Frontiers in microbiology 5, 1-9.

Zak, D., Gelbrecht, J., 2007. The mobilisation of phosphorus, organic carbon and ammonium in the initial stage of fen rewetting (a case study from NE Germany). Biogeochemistry 85, 141-151.

Zak, D., Kleeberg, A., Hupfer, M., 2006. Sulphate-mediated phosphorus mobilization in riverine sediments at increasing sulphate concentration, River Spree, NE Germany. Biogeochemistry 80, 109-119.

Zaman, M., Blennerhassett, J. D., 2010. Effects of the different rates of urease and nitrification inhibitors on gaseous emissions of ammonia and nitrous oxide, nitrate leaching and pasture production from urine patches in an intensive grazed pasture system. Agriculture, Ecosystems & Environment 136, 236-246.

Zedler, J. B., Kercher, S., 2005. Wetland resources: status, trends, ecosystem services, and restorability. Annual Review of Environment and Resources 30, 39-74.

Zhang, L., Keller, J., Yuan, Z., 2009. Inhibition of sulfate-reducing and methanogenic activities of anaerobic sewer biofilms by ferric iron dosing. Water Research 43, 4123-4132.

Zhao, F., Xi, S., Yang, X., Yang, W., Li, J., Gu, B., He, Z., 2012. Purifying eutrophic river waters with integrated floating island systems. Ecological Engineering 40, 53-60.

Zhu, G., Jetten, M. S. M., Kuschk, P., Ettwig, K. F., Yin, C., 2010. Potential roles of anaerobic ammonium and methane oxidation in the nitrogen cycle of wetland ecosystems. Applied Microbiology and Biotechnology 86, 1043-1055.

Zhuang, Q., Melillo, J. M., Kicklighter, D. W., Prinn, R. G., McGuire, A. D., Steudler, P. A., Felzer, B. S., Hu, S., 2004. Methane fluxes between terrestrial ecosystems and the atmosphere at northern high latitudes during the past century: A retrospective analysis with a process-based biogeochemistry model. Global biogeochemical cycles 18, GB3010.

Summary

Wetlands are often referred to as "*the kidneys of the landscape*" due to multiple ecosystem services they provide regarding water, such as flood mitigation and the improvement of water quality, and as "*biological supermarkets*" due to the extended food webs and rich diversity they support. At a global scale, however, many wetlands have been lost or degraded due to human development including drainage of land for agricultural purposes, construction of dams for hydropower generation or for the creation of water reservoirs, and eutrophication and pollution. To compensate for the loss and degradation of natural wetlands, drained wetlands are now being restored and new wetlands are being created. Furthermore, wetlands are specifically constructed for nutrient removal and carbon (C) sequestration. Essentially, all these different wetland ecosystems show similar biogeochemical and ecological mechanisms, including plant nutrient uptake, soil precipitation and adsorption, iron oxidation and reduction, photosynthesis, decomposition, and methane (CH_4) production and oxidation. In addition, wetland soils and vegetation, major components of wetlands, play an important role in the biogeochemical cycling of C and nutrients, and resulting ecosystem services. The main goal of this thesis was to study and optimize nutrient uptake and C sequestration in macrophyte-dominated wetlands (Chapter 1).

To achieve this, a series of laboratory and mesocosm experiments were carried out to study the interacting roles of soil and vegetation type. We focused on both biological and biogeochemical processes related to wetland services including water storage, flood abatement, nutrient removal, C sequestration, and soil phosphorus (P) mining.

The construction and restoration of permanent or riparian (temporarily flooded) wetlands plays a central role in climate-adaptive water management, because of the water storage and flood protection services they provide. However, because projects are often planned on former agricultural lands, excess P stored in these soils may lead to eutrophication problems and may thus severely compromise these services, due to high rates of P release to the overlying water and surroundings. It is therefore urgent for water management to find an easy tool to predict P release rates after short-term flooding that is widely applicable to a range of different soil types. In Chapter 2, we combined experimental work on P mobilization rates with the measurement of a suite of soil characteristics for a large set of different soils ranging from sandy to highly organic. Multi-model inference showed that water-extractable P is an excellent predictor, explaining 86.9% of the variation. In addition, we determined temperature dependence of P-mobilization, to be able to correct for temperature related seasonal variations. Increasing the temperature from 8 to 18°C almost tripled P mobilization rates, but the variation could not be linked to any of the soil characteristics measured. In this way we provide a widely-applicable, very easy and cost-effective tool to predict P mobilization rates during short-term flooding, which is essential for decision support in water management related to wetland creation for water storage and other services. The outcome can be used as a standard method to support water, land and nature managers in their decision-making concerning the optimal location for the construction of and restoration of wetlands.

Nutrient sequestration from surface waters by aquatic macrophytes and soils provides an important service of both natural and constructed wetlands. So far, however, the focus has almost entirely been on emergent (helophyte) species, while the use of submerged and floating macrophytes may be more efficient since these filter nutrients directly from the surface water. It remains unclear, however, whether their efficiency is sufficient for wastewater purification, and how efficient different submerged or floating macrophytes are under different nutrient loads and on different soil types. We therefore used a full factorial outdoor mesocosm design to test the effects of different nutrient loadings on the resulting distribution of nutrients over different compartments: water layer, plant biomass and soil (Chapter 3). In the experiment we compared different plant species (*Ceratophyllum demersum*, *Chara hispida*, *Myriophyllum spicatum* and *Azolla filiculoides*) in combination with different soil types (peat, peaty clay and clay soils). Our results clearly show that aquatic macrophytes can be used for wastewater polishing, but not as a stand-alone purification treatment for nutrient removal from wastewater. At loads $\leq$ 22 mg P m^{-2} d^{-1}, *M. spicatum* is the best option, whereas at loads $\geq$ 22 mg P m^{-2} d^{-1}, *A. filiculoides* removes P more efficiently. The outcome of the results enables the selection of efficient macrophytes species for wastewater polishing depending on nutrient loading rates, which is essential for decision support in water management using constructed wetlands for nutrient removal by biomass harvesting. Furthermore, we have shown that soil type is a previously underestimated factor influencing the efficiency of nutrient removal and immobilization.

In addition to nutrient removal, macrophytes, such as water hyacinth (*Eichhornia crassipes*), also play an important role in the regulation of greenhouse gas (GHG) fluxes. There is, however, controversy regarding the net effect of water hyacinth vegetation: do they act as GHG sources or sinks? We hypothesized that the differential effects of water hyacinth mats on GHG fluxes may be due to variation in plant density and whether or not the plants are rooted in the sediment. We therefore used a full-factorial, controlled indoor aquarium experiment aiming to elucidate the effects of plant density and sediment rooting on the nutrient dynamics and GHG fluxes of water hyacinth vegetation (Chapter 4). In accordance with our expectations, we found that GHG fluxes were strongly influenced by plant density and rooting. Water hyacinth presence significantly increased diffusive CH_4 fluxes. These fluxes increased with plant density and, especially when plants were rooting in the sediment. Due to water hyacinth's high carbon dioxide (CO_2) sequestration rates, the overall GHG budget in terms of CO_2 equivalents still resulted in water hyacinth mats being near-neutral or even a GHG sink, depending on water hyacinth density. This indicates that, when assessing regional GHG fluxes for wetlands, not only plant presence but also its density and water depth – regulating sediment-root contact – should be taken into account.

Hypertrophic shallow wetlands are either dominated by algae or cyanobacteria, or covered by floating macrophytes such as water ferns (*Azolla* spp.), and may show high GHG emissions. In addition, it is well known that P can easily become mobilized from soils to the overlying water

after the restoration and construction of wetlands on former agricultural lands including drained peatlands. Here, the active removal of P by the growth and harvest of fast growing *Azolla* spp. may be a viable scenario to avoid downstream eutrophication problems, recycle excess P, and decrease GHG emissions. Although it is well known that iron (Fe) is a key factor determining biogeochemical processes of wetlands, the exact factors and mechanisms regulating P, GHG and total organic carbon (TOC) emissions from *Azolla* dominated wetlands remain unclear. We therefore determined the mobilization of Fe and P into the water layer, their assimilation by *A. filiculoides*, and GHG and TOC emissions for two rewetted peat soils with different Fe reduction rates, in a full factorial, controlled aquarium experiment (Chapter 5). We showed that electron donor availability (organic matter fractions), rather than Fe and P availability, could explain Fe reduction rates, which in turn strongly regulate the emissions of GHG, TOC and P from *Azolla* dominated wetlands. Although the cover of the water layer by *Azolla* leads to C, P and Fe sequestration, rewetted peat soils may still act as C and GHG sources due to their high anaerobic decomposition rates. The outcome of the results is not only important to understand the biogeochemistry of *Azolla*-dominated wetlands, but also for the application of *Azolla* sp. to recycle P from nutrient loaded agricultural soils after rewetting.

Finally, in Chapter 6, a conceptual design is proposed to optimize wetland ecosystem services including flood abatement (water retention and water storage), soil P mining, wastewater treatment and C sequestration by biomass production, based on the synthesis of the results of the laboratory and mesocosm experiments described in the previous chapters. In addition, the implications for water management, land management, and resource management are elaborated on in this chapter.

Samenvatting

Wetlands worden vaak omschreven als “de nieren van een landschap” vanwege de veelheid aan watergerelateerde diensten die ze aan het ecosysteem bieden, zoals het verminderen van overstroming en de verbetering van waterkwaliteit, en ook als “biologische supermarkten” vanwege de uitgebreide voedselwebben en rijke diversiteit die ze mogelijk maken. Helaas zijn veel wetlands op mondiale schaal verloren gegaan of achteruitgegaan door menselijke ingrepen zoals drainage van het land voor landbouwdoeleinden, constructie van dammen voor het opwekken van energie of voor het aanleggen van waterreservoirs, en ook door eutrophiëring en verontreiniging. Om voor het verlies en de beschadiging van natuurlijke wetlands te compenseren, worden gedraineerde wetlands hersteld en nieuwe wetlands gecreëerd. Verder worden wetlands ook specifiek aangelegd voor nutriëntverwijdering en het vastleggen van koolstof (C). In essentie hebben al deze verschillende wetlandecosystemen vergelijkbare biogeochemische en ecologische mechanismen zoals nutriëntopname door planten, neerslag en adsorptie van nutriënten aan het sediment, ijzeroxidatie en reductie, fotosynthese, decompositie en methaan (CH_4) productie en oxidatie. Bovendien spelen de bodems en vegetatie, belangrijke componenten van wetlands, een belangrijke rol in de biogeochemische kringloop van C en nutriënten en de daaruit resulterende ecosysteemdiensten. Het voornaamste doel van dit proefschrift was het bestuderen en optimaliseren van nutriëntopname en koolstofvastlegging in macrofyt-gedomineerde wetlands (Hoofdstuk 1).

Om dit te bereiken werd een serie laboratorium- en mesocosmexperimenten uitgevoerd om de rol van zowel bodem- als vegetatietype te bestuderen en werd gefocust op zowel biologische als biogeochemische processen die relateren aan deze wetlanddiensten zoals wateropslag, verminderen van overstroming, nutriëntverwijdering, C vastlegging en het oogsten van bodemfosfor (P).

De aanleg en het herstellen van permanente of tijdelijk overstroomde wetlands speelt een belangrijke rol in klimaatbewust watermanagement, vanwege de wateropslag en de bescherming tegen overstroming die ze bieden. Echter, omdat projecten vaak gepland worden op vroegere landbouwgebieden, kan het overvloedige fosfor opgeslagen in deze bodems leiden tot eutrofiëringsproblemen en zo deze diensten ernstig ondermijnen door de snelle P afgifte aan het bovenliggende water en de omgeving. Daarom is het uiterst belangrijk voor watermanagement om een eenvoudig middel te vinden voor het voorspellen van P afgifte na kortdurende overstroming, dat breed toepasbaar is op een scala aan verschillende bodemtypen. In hoofstuk 2 combineerden we experimenteel werk aan P mobilisatiesnelheden met het meten van een ruime variatie aan bodemeigenschappen voor een grote set aan bodemtypen variërend van zandig tot vooral organisch. Multi-model inferentie liet zien dat water-extraheerbare P een uitstekende voorspeller is van P-mobilisatie die 86.9% van de variatie verklaart. Verder hebben we de temperatuursafhankelijkheid van P-mobilisatie bepaald om te kunnen corrigeren voor temperatuurgerelateerde seizoensvariaties. Het verhogen van de temperatuur van 8 naar 18°C verdrievoudigde haast de P-mobilisatiesnelheden, maar de variatie kon niet aan enige gemeten bodemeigenschap gerelateerd worden. Op deze manier voorzien we in een breed toepasbaar, zeer eenvoudig en kosteneffectief middel om P-mobilisatiesnelheden te voorspellen tijdens kortdurende overstro-

ming, dat essentiëel is voor het ondersteunen van beslissingen in watermanagement aangaande het creëren van wetlands voor wateropslag en andere diensten.

Het vastleggen van nutriënten uit oppervlaktewateren door aquatische macrofyten en bodems is een belangrijke dienst van zowel natuurlijke als aangelegde wetlands. Tot nu toe is de focus echter bijna geheel op helofyten (emergente planten) gelegd, terwijl het gebruik van ondergedoken en drijvende macrofyten efficiënter zou kunnen zijn, omdat deze de nutriënten direct uit het oppervlaktewater filteren. Het is echter nog onduidelijk of hun efficiëntie voldoende is voor afvalwaterzuivering en hoe efficiënt verschillende ondergedoken of drijvende planten zijn bij verschillende nutriëntbelasting en op verschillende bodemtypen. We hebben daarom een volledig gekruist mesocosm ontwerp in de openlucht gebruikt om de effecten van verschillende nutriëntbelasting op de resulterende verdeling van nutriënten over de verschillende compartimenten te bepalen: waterlaag, plantenbiomassa en bodem (Hoofdstuk 3). In het experiment vergeleken we verschillende plantensoorten (*Ceratophyllum demersum*, *Chara hispida*, *Myriophyllum spicatum* en *Azolla filiculoides*) in combinatie met verschillende bodemtypen (veen, veenachtig klei en klei bodem). Onze resultaten laten duidelijk zien dat aquatische macrofyten gebruikt kunnen worden voor het lage bereik van de afvalwaterzuivering (polishing), maar niet als op zichzelfstaande zuiveringsbehandeling voor nutriëntverwijdering uit afvalwater. Bij belasting $\leq$ 22 mg P m^{-2} d^{-1} is *M. spicatum* de beste optie, terwijl bij belasting $\geq$ 22 mg P m^{-2} d^{-1} *A. filiculoides* efficiënter P verwijdert. De uitkomst van de resultaten maakt de selectie mogelijk van macrofytsoorten efficiënt voor afvalwaterzuivering afhankelijk van nutiëntbelastingsnelheid, die essentiëel is voor het ondersteunen van beslissingen in watermanagement en gebruikmaakt van aangelegde wetlands voor nutriëntverwijdering door het oogsten van biomassa. Verder hebben we aangetoond dat bodemtype een voorheen onderschatte factor is die grote invloed heeft op de efficiëntie van nutriëntverwijdering en immobilisatie.

Behalve nutriëntverwijdering, spelen macrofyten zoals de waterhyacint (*Eichhornia crassipes*) ook een belangrijke rol in de regulatie van broeikasgasfluxen. Er is echter controversie over het netto effect van waterhyacint-vegetatie als bron van broeikasgassen of juist als vastlegger. Onze hypothese was dat het variërende effect van matten van waterhyacint op fluxen van broeikasgassen het gevolg zou kunnen zijn van variatie in plantdichtheid en of de planten in het sediment geworteld zijn. Daarom hebben we een volledig gekruist, gecontroleerd, binnenshuis aquariumexperiment gedaan met als doel de effecten van plantdichtheid en bodemworteling op de dynamica van nutriënten en fluxen van broeikasgas in vegetatie van waterhyacint op te helderen (Hoofdstuk 4). In overeenstemming met onze verwachting vonden we dat fluxen van broeikasgas sterk beïnvloed werden door plantdichtheid en worteling. De aanwezigheid van waterhyacint verhoogde de diffusieve CH_4 fluxen signifcant. Deze fluxen namen toe met plantdichtheid, vooral wanneer planten in het sediment wortelden. De hoge snelheid waarmee waterhyacint CO_2 vastlegt, zorgde er echter in het totale budget van broeikasgassen, gemeten in CO_2 equivalenten, voor dat matten van waterhyacint nog steeds bijna neutraal waren of zelfs broeikasgas vastlegden, afhankelijk van

de dichtheid van waterhyacint. Dit wijst erop dat, bij het inschatten van regionale fluxen van broeikasgas, niet alleen de aanwezigheid van planten, maar ook de dichtheid en waterdiepte – die het contact tussen sediment en wortels reguleert, beschouwd moet worden.

Hypertrofe ondiepe wetlands worden ofwel gedomineerd door algen of cyanobacteriën of bedekt door drijvende macrofyten zoals kroosvarens (*Azolla* spp.) en kunnen hoge emissies van broeikasgassen vertonen. Daarenboven is het welbekend dat P eenvoudig gemobiliseerd kan worden vanuit bodems naar het bovenliggende water na de restauratie en aanleg van wetlands op vroegere landbouwgrond inclusief drooggelegde veengronden. Hier kan het actief verwijderen van P door de groei en oogst van snelgroeiende *Azolla* spp. een realistisch scenario zijn om stroomafwaarts eutrofiëringsproblemen te voorkomen, overtollig P te recyclen en de uitstoot van broeikasgassen te verminderen. Hoewel het welbekend is dat ijzer (Fe) een sleutelfactor is in het bepalen van biogeochemische processen van wetlands, blijven de exacte factoren en mechanismen die uitstoot van P, broeikasgassen en totale organische koolstof (TOC) uit *Azolla* gedomineerde wetlands reguleren nog onduidelijk. We hebben daarom de mobilisatie van Fe en P naar de waterlaag, hun assimilatie door *A. filiculoides*, en uitstoot van broeikasgassen en TOC bepaald voor twee opnieuw vernatte veenbodems met verschillende Fe reductiesnelheden in een volledig gekruist gecontroleerd aquariumexperiment (Hoofdstuk 5). We lieten zien dat beschikbaarheid van geschikte electronendonoren, (organische verbindingen) meer dan Fe en P beschikbaarheid, de Fe reductiesnelheiden konden verklaren die op hun beurt sterk de emissie van broeikasgas, TOC en P vanuit door *Azolla* gedomineerde wetlands reguleren. Hoewel de bedekking van de waterlaag door *Azolla* tot vastlegging van C, P en Fe leidt, kunnen opnieuw vernatte veenbodems nog steeds een bron van C en broeikasgas zijn vanwege hun hoge hoeveelheid anaerobe decompositie. De uitkomst van de resultaten is niet alleen belangrijk voor het begrijpen van de biogeochemie van door *Azolla* gedomineerde wetlands, maar ook voor de toepassing van *Azolla* spp. in het recyclen van P uit nutriëntbelaste landbouwgronden na het opnieuw vernatten.

Tot slot, in Hoofdstuk 6, stellen we een conceptueel ontwerp voor om de ecosysteemdiensten van wetlands zoals het verminderen van overstroming (water vasthouden en wateropslag), het oogsten van bodemfosfor, behandeling van afvalwater en het vastleggen van C door biomassaproductie te optimaliseren, gebaseerd op de integratie van de resultaten van de laboratorium- en mesocosmexperimenten zoals beschreven in voorgaande hoofdstukken. Verder worden de implicaties voor watermanagement, landmanagement, en grondstofmanagement uitgelegd in dit hoofdstuk.

摘要 (Chinese summary)

湿地常常由于其提供的与水有关的多样的生态服务功能被称为“地球之肾”，例如防洪和水质的改善，并且由于其所支撑的巨大的食物网和丰富的生物多样性被称为“生物超市”。然而由于人类的发展包括排干湿地发展农业，水电大坝建设和水库大坝建设，富营养化和污染，在全球范围内很多湿地正在消失或者退化。为了弥补自然湿地的消失和退化，排干的湿地正在被修复，并且新的湿地正在被建立。此外，湿地尤其是为了营养物去除和碳固定而构建的。在本质上，所有不同的湿地生态系统具有相似的生物地球化学和生态学机制，包括植物的营养吸收，土壤的沉淀和吸附，铁的氧化和还原，光合作用，降解，以及甲烷的生成和氧化。另外，湿地的重要组成部分：湿地土壤和植物，在碳和营养物的生物地球化学循环和由此引发的生态系统服务功能中起着重要的作用。这本论文主要目标是研究和最大化大型植物占主导的湿地中的营养物去除和碳固定（第一章）。

为了实现这一目标，一系列的室内和围隔实验被用来研究土壤和植物类型的交互作用。我们聚焦于与湿地功能相关的生物和生物地球化学过程，包括防洪，营养物去除，碳固定和土壤磷（P）开采。

由于永久的或者河边的（短暂地淹没的）湿地可以提供水储存和防洪的功能，因此构建和恢复这种湿地在气候调控的水管理中起着重要的作用。然而，由于这些项目经常规划在废弃的农业用地上，储存于这些土壤中过量的磷会由于较高的释放率被释放到上覆水和周围的环境中，从而可能会导致富营养化问题，因此可能会危害湿地的这些功能。因此水管理者急于找到可以广泛地应用于很多不同土壤类型的简单工具去预测短期淹没后磷的释放率。在第二章，我们把实验所得的磷的释放率和来自于不同土壤类型从沙质土到高度地有机土的一系列的土壤特征结合了起来。多模型推理表明水提取的磷是一个极好的预测指标，能解释 86.9% 的变化。另外，为了能够校正与温度相关的季节变化，我们确定了磷释放率的温度依赖。在 18°C 时磷的释放率几乎是 8°C 时的三倍，但是这种变化不能与所测量的任何一个土壤特征联系起来。我们用这种方式提供了一个广泛应用的，很容易的，并且划算的工具去预测短期土壤淹没后磷的释放率，这对与湿地创建（为了水储存和其他功能）有关的水管理中的决策支持起着至关重要的作用。研究结果可以被水，土地和自然管理者用作标准方法去决定构建和恢复湿地的最佳地点。

自然和人工湿地中水生大型植物和土壤对表面水中营养物的固定是自然和人工湿地的一个重要的服务功能。然而到目前为止研究几乎全部聚焦在挺水（沼生）植物，而由于沉水和漂浮植物能够直接过滤表面水中的营养物，因此使用沉水植物和漂浮植物可能更加有效。但是，是否它们的效力足以废水净化，并且不同的沉水或者漂浮植物在不同营养物负荷下和在不同土壤类型上的效果仍然不可知。因此我们使用了一个全因子室外围隔设计去测试不同营养物负荷对营养物在水层，植物生物量和土壤中分布的影响（第三章）。在实验中我们把不同的植物物种（金鱼藻，轮藻，穗状狐尾藻以及细叶满江红）与不同的土壤类型（泥炭土，泥炭粘土，粘土）结合起来比较。我们的结果清楚地表明水生大型植物可以被用作进一步的废水处理，不能作为独立的净化

处理去去除废水中营养物。当负荷 ≤ 22 mg P m^{-2} d^{-1} 的时候，穗状狐尾藻是最好的选择，当负荷 ≥ 22 mg P m^{-2} d^{-1} 的时候，细叶满江红能更有效地去除磷。研究结果使得我们能够依据营养物负荷来选择有效的大型植物物种用于进一步的废水处理，这对于水管理中利用人工湿地经由生物量的采收去除营养物的的决策支持至关重要。另外，我们表明土壤类型是之前低估的影响营养物去除和固定效率的重要因素。

除了去除营养物，大型植物，例如凤眼莲，同样管理着温室气体的流量。然而，关于凤眼莲对温室气体的净作用还是存在着争议：它们是温室气体源还是汇？我们假定凤眼莲对于温室气体的流量的不同作用可能取决于植物密度的不同以及是否植物扎根于沉积物。因此我们使用一个全因子的，控制室内水族箱实验去阐明植物密度和沉积物扎根对营养物动态和温室气体流量的影响（第四章）。与我们的期望一致，我们发现植物密度和沉积物扎根强烈地影响着温室气体的流量。凤眼莲的出现显著地增加了扩散的甲烷流量。这些流量随着植物密度而增加，尤其当植物扎根于沉积物时。由于凤眼莲高的二氧化碳固定率，凤眼莲对温室气体的净作用（依据二氧化碳等价物）依赖于植物密度是近乎中性或者是温室气体的汇。这指出当为湿地评估地区性的温室气体流量时，不仅仅是植物的出现，它的密度和水的深度-管理着沉积物-根的接触-应该被考虑在内。

高营养的浅湿地要么被绿藻或者蓝藻占领，要么被漂浮植物所覆盖，例如水蕨（满江红），并且可能具有高的温室气体排放。此外，众所周知，在废弃的农业土包括排干的泥炭地上构建和恢复湿地后，磷会很容易的从土壤中释放到上覆水中。在这里通过迅速增长的满江红的生长和采收来有效地去除磷可能是一个避免下游富营养化问题，循环过量的磷以及降低温室气体排放的可行方案。尽管众所周知铁是决定湿地生物地球化学过程的重要因素，在满江红占主导地位的湿地中具体管理磷，温室气体，有机碳排放的因素和机制仍然不清楚。因此我们用全因子，控制水族箱实验决定了铁和磷到水层的释放，它们被细叶满江红的吸收，以及温室气体和有机碳的排放（第五章）。本实验我们用了两种淹没的泥炭土，它们具有不同的铁还原率。我们表明电子捐献者的可获得性（有机物部分）而不是铁和磷的可获得性解释了铁的还原率。铁的还原率反过来强烈地管理着满江红占主导地位湿地中温室气体，总有机碳以及磷的排放。尽管水层满江红的覆盖会引发碳，磷和铁的固定，由于它们高的厌氧降解率，淹没的泥炭土仍然作为碳和温室气体的源。研究的结果不仅对于理解满江红占主导地位的湿地的生物地球化学循环具有重要的意义，并且能够在土壤淹没后利用满江红来循环利用农业土中的磷。

最后，在第六章我们基于整合前面几章的室内和围隔实验的研究结果，提出了最大化湿地生态系统服务功能的设计方案。湿地的生态系统服务功能包括防洪（水停留和水储存），土壤磷开采，废水处理以及生物量产出的碳固定。此外，在本章详细说明了研究结果对于水管理，土地管理以及资源管理的意义。

Acknowledgements

On 8 October 2012 I visited the Institute for Water and Wetland Research for the first time, and it was my fortune to meet a lot of great people there. I really enjoyed my time with them, and they gave me a lot of help and support during the last four years. Here, I would like to express my thanks to all these wonderful people.

First and foremost, I would like to express my deepest gratitude to my (co-) promotors who offered me the chance to perform scientific research on constructed wetlands. Leon, thank you very much for your expert guidance, unfailing support, great patience and encouragement throughout my research and writing. In the preparation of this thesis, you have spent much time reading my thesis and provided me with inspiring advise. Without your professional and patient instruction, the completion of this thesis would have been impossible. Fons, you have provided me with constructive suggestions, valuable ideas, and insightful criticism in every stage of my research and the writing of this thesis. Those comments were all very valuable and helpful for improving my experiments, as well as guiding my research and writing my manuscripts. Jan, thank you very much for your involvement in most of my experiments. Your profound teaching provided a sound theoretical and experimental basis for the design of my experiments. Monique, my daily supervisor, you always offered me a helping hand, which I appreciate enormously. Your encouragement and unwavering support has definitely stimulated my enthusiasm for working on my research and thesis!

I am also indebted to all my MSc students: Johan, Andreas, Han, Sanne, and Daniel, who did their internships with me. You have been essential for the implementation of the experimental work and data acquisition, and without your help I would still be doing practical work by now. This also holds for my former colleagues Laury and Evi. Thank you all very much for your contribution to my thesis. Sarah Faye and Ernandes, I would like to thank you both very much for our close and pleasant collaboration while working on our joint manuscripts. Besides, my gratitude also goes to Jelle Eygensteyn, Paul van der Ven and Sebastian Krosse who measured a large number of samples and provided technical advise and support. I would also like to thank Peter Cruijsen for his help to collect soils in the field, and Ankie De Vries-Brock, Germa Verheggen and Roy Peters for their assistance with chemical analyses. Most of my experiments were performed in the greenhouse facilities, and therefore I wish to express my thanks to Gerard for providing his expertise and experimental space, and Walter, Harry, Yvette for looking after my water hyacinths and *Azolla* plants in the greenhouse facilities.

I would also like to pay tribute to all department members who have given me great support. I really liked the atmosphere at the department and I will definitely miss the time we spent together. Tjisse, thank you for helping me with data analyses; I learned a lot from you. Sarian, Ernandes and Ralf, thank you so much for your assistance with the greenhouse gas flux measurements, calculations and our joint manuscript writing. Dries, thank you for designing my first pilot experiment. Marlous and Ralf, thank you for playing Pokémon Go with me; I enjoyed it a lot.

Eva and Ernandes, thank you for helping me to prepare beer and snacks for my farewell party. Ralph, thank you for introducing me to this nice website where I can download professional books. Sarah Faye and Annieke, thank you very much for inviting me to your apartments for dinner. Valérie and Tamara, thank you for always being so nice to me and lending me your ears. Gijs, Evi, Moni, and José, thank you for helping me to collect the soils in the Netherlands, and Laura for borrowing your field equipment. Jeroen, Christian and Leon vd B, thank you for spending your time listening to me and helping me to solve my practical problems. I would like to express my special thanks to José B, who arranged my insurance, many other documents, and so much more for me.

Besides, I am also grateful to people in other departments. Marjolein and Marco, thank you very much for your help with statistics. Maartje, thank you for inviting me to join the Viking Run (Mud Version); I had a lot of fun. Hans, Eric, Heidi, Hannie, Annemiek, Jan Willem, Niels, Anne, Dina, Nils, Onno, Nicky, Isabela, Stuart, Marloes, Elke, Wilco, Casper and Janneke, thank you for all interesting conversations we had during coffee break, lunch break and Friday *borrels*. Jiemeng, Hanjing and Qian, thank you very much for your company and encouragement. Natan, thank you for teaching me how to use R, helping me to improve my English, playing board games with me, and translating my English summary into Dutch. Emma, thank you for letting me live in your place for three weeks, cooking delicious food for me, and playing squash with me, and I really do appreciate this.

最后我想在这里感谢我生命中最重要的人—我的爸爸、妈妈、老公阎云升 (Steven)、公公、婆婆。正是由于你们一贯的理解和支持我才能在异国他乡开始我的博士生涯；正是由于你们的鼓励和帮助我才能顺利地完成我的学业和博士毕业论文。在荷四年，我对你们的陪伴真的很少，但你们从来没有一句怨言。我真的很感谢你们对我的无条件地付出和关爱。你们永远是我最强大的精神支柱和后援团，谢谢你们，我爱你们！

唐莹莹/Yingying

Curriculum Vitae
Publications

Curriculum Vitae

Yingying Tang (唐莹莹) was born on July 5, 1984 in Luan, Anhui province, China. She attended primary, middle and high schools in her hometown. In 2003, she started to study Environmental Science at Anhui Normal University in Wuhu, Anhui province, China. In 2007, she received her bachelor degree and after that she worked as a business assistant for a company. However, she then realized that she was still more interested in ecology than in business, and therefore decided to quit her job and start to prepare for her postgraduate entrance examination. In 2009, she passed this exam and started to study ecology at East China Normal University, in Shanghai. As a graduate student, she carried out research on nitrogen removal mechanisms of ecological floating-beds, and received her master's degree in 2012. After that, she was able to obtain a scholarship from the China Scholarship Council and then went to the Netherlands for her PhD study. During her PhD study, she worked on the key biogeochemical processes in macrophyte-dominated wetlands that determine various wetland services. The results of her research are elaborated in this thesis.

Publications

1. Tang Y., Van Kempen M.M.L., Van der Heide T., Manschot J.J.A., Roelofs J.G.M., Lamers L.P.M., Smolders A.J.P. (2016). A tool for easily predicting short-term phosphorus mobilization from flooded soils. Ecological Engineering 94: 1-6.

2. Tang, Y., Harpenslager, S.F., van Kempen, M.M.L., Verbaarschot, E.J.H., Loeffen, L.M.J.M., Roelofs, J.G.M., Smolders, A.J.P., Lamers, L.P.M. (2016). Aquatic macrophytes can be used for wastewater polishing, but not for purification in constructed wetlands, Biogeosciences Discuss., doi:10. 5194/bg-2016-80.

3. Sobreira Oliveira Junior, E., Tang, Y., van den Berg, S.J.P., Lamers, L.P.M., Kosten, S. (2016). Rooting and plant density strongly determine greenhouse gas budget of water hyacinth (*Eichhornia crassipes*) mats, Biogeosciences Discuss., doi:10.5194/bg-2016-297.

4. Tang Y., Van Kempen M.M.L., Roelofs, J.G.M., Kosten, S., Smolders, A.J.P., Lamers, L.P.M. The roles of iron biogeochemistry and *Azolla* growth in the regulation of greenhouse gas, organic carbon and phosphorus emissions from rewetted peat soils, *submitted*.